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CARCINOGENESIS ABSTRACTS

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PREFACE

Carcinogenesis Abstracts is a publication of the National Cancer Institute. The journal serves as a vehicle through which current documentation of carcinogenesis research highlights are compiled, condensed, and disseminated on a regular basis. It represents an integral part of the Institute's program of fostering and supporting coordinated research into cancer etiology. Issues of *Carcinogenesis Abstracts* normally contain three-hundred abstracts and three-hundred citations (unaccompanied by corresponding abstracts). Abstracts and citations refer to the current scientific literature that describes the most significant carcinogenesis research carried on at the National Cancer Institute, other governmental agencies, and private institutions. *Carcinogenesis Abstracts* is intended to be a highly useful current awareness tool for scientists engaged in carcinogenesis research or related areas. The great number and diversity of publications relevant to carcinogenesis make imperative the availability of this service to investigators whose work requires that they keep abreast with current developments in the field.

Carcinogenesis Abstracts is normally published monthly. Volume XII covers the scientific literature published from Jan 1974 through Dec 1974. To increase the usefulness of *Carcinogenesis Abstracts*, Volume XII, a Wiswesser Line Notation index and a Chemical Abstracts Service Registry Number index have been provided. These indexes reference compounds described in abstracted articles. A cumulative subject, author, CAS Registry Number, and Wiswesser Line Notation index for Volume XII will be published shortly after the final regular issue.

Carcinogenesis Abstracts is available free of charge to libraries and to individuals who have a professional interest in carcinogenesis. Requests for *Carcinogenesis Abstracts* from qualified individuals should include statements of their relationship to carcinogenesis research. All correspondence should be addressed as follows.

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NOTE

Journal names are abbreviated according to the list of abbreviations used by *Index Medicus*. For journals not covered by *Index Medicus*, the abbreviations found in *Chemical Abstracts Service Source Index, 1907-1974 Cumulative*, are used. New journals are verified in *New Serial Titles* and abbreviated according to *International Standard ISO 833*. An asterisk indicates the author to address (other than the primary) in requesting reprints.

LANGUAGE ABBREVIATIONS

Afr.	Afrikaans	Ind.	Indonesian
Ara.	Arabic	Ita.	Italian
Bul.	Bulgarian	Jpn.	Japanese
Chi.	Chinese	Kor.	Korean
Cro.	Croation	Lav.	Latvian
Cze.	Czech	Lit.	Lithuanian
Dan.	Danish	Nor.	Norwegian
Dut.	Dutch	Pol.	Polish
Eng.	English	Por.	Portuguese
Est.	Estonian	Rum.	Rumanian
Fin.	Finnish	Rus.	Russian
Fle.	Flemish	Ser.	Serbo-Croatian
Fre.	French	Slo.	Slovak
Geo.	Georgian	Spa.	Spanish
Ger.	German	Swe.	Swedish
Gre.	Greek	Tha.	Thai
Heb.	Hebrew	Tur.	Turkish
Hun.	Hungarian	Ukr.	Ukrainian
Ice.	Icelandic	Vie.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

A	angstrom(s)
ACTH	adrenocorticotropic hormone
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BCG	Bacillus Calmette Guerin
bid	twice daily
C	degrees centigrade
cal	calorie(s)
kcal	kilocalorie(s)
cc	cubic centimeter(s)
Ci	curie(s)
mCi	millicurie(s)
μCi	microcurie(s)
cm	centimeter(s)
CNS	central nervous system
cpm	counts per minute
dl	deciliter(s)
ml	milliliter(s)
μl	microliter(s)
DNA	deoxyribonucleic acid
ED_{50}	median effective dose
EDTA	ethylenediamine tetraacetic acid
ESR	erythrocyte sedimentation rate
g	gram(s)
kg	kilogram(s)
mg	milligram(s)
μg	microgram(s)
Hb	hemoglobin
hr	hour(s)
ia	intra-arterial
ic	intracerebral
icav	intracavitary
id	intradermal
ILS	increased life span
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratumorous
IU	International Unit
iv	intravenous
K_m	Michaelis constant
LD	lethal dose
LD_{50}	median lethal dose
m	meter(s)
mm	millimeter(s)
M	molar
mM	millimolar
μM	micromolar
mOsm	milliosmolar
mEq	milliequivalents
min	minute(s)
mo	month(s)
MTD	maximum tolerated dose
N	normal concentration
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotidephosphate
NADPH	reduced nicotinamide adenine dinucleotide-phosphate
ng	nanogram(s) (10^{-9})
od	once daily
Pa	ambient pressure
PAS	periodic acid-Schiff
pg	picogram(s) (10^{-12})
pgEq	picogram equivalent
po	orally
ppb	parts per billion
ppm	parts per million
qid	four times daily
qod	every other day
QO_2	oxygen quotient
R	roentgen(s)
RBC	red blood cells (erythrocytes)
RNA	ribonucleic acid
sc	subcutaneous
sec	second(s)
SGOT	serum glutamic-oxalacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SRBS	sheep red blood cells
TCD	tissue culture dose
TCD_{50}	median tissue culture dose
tid	three times daily
U	unit(s)
mU	milliunit(s)
UV	ultraviolet
vol	volume
WBC	white blood cells (leukocytes)
wk	week(s)
wt	weight
x	times
yr	year(s)

CONTENTS

0001 ALCOHOLISM AND THE RISK OF CANCER. (Eng.) Lowenfels, A. B. (New York Med. Coll., Valhalla, N. Y.). *Ann. N.Y. Acad. Sci.* 252:366-373; 1975.

A review of the accumulated evidence linking alcohol and cancer was presented. Possible factors predisposing neoplasm formation include the dubious carcinogenic effect of ethanol, contamination of alcoholic beverages, alcoholic damage to mucus membranes, enhancement of the effect of smoking, and an associated nutritional defect in the alcoholic. Reports underscore a strong relation betwen alcohol and oropharyngeal tumors, especially after exposure to "hard liquor"; likewise, cancer of the larynx is associated with consumption of whiskey. Whereas excess drinking does not lead to parotid tumors, there was a strong correlation with the incidence of esophageal cancer, due in part to a synergistic effect with smoking; the type of beverage and source of carbohydrate are significant in this case. World cancer statistics show a relationship of the frequency of stomach cancer to the frequency of esophageal cancer, with cancer of the cardiac portion most common. Hepatomas almost always arise in a previously damaged liver of which, in Western countries, alcoholic cirrhosis is the commonest antecedent. Chronic pancreatitis, a frequent disease in alcoholics, is a common finding in patients with pancreatic cancer; despite a greater incidence of alcoholics found dying from pancreatic cancer, the link between alcoholism and subsequent death still remains controversial. No significant correlation is found between alcoholism and cancer of the prostate. However, a general alcohol intolerance is noted in cancer patients, with symptoms (pain, discomfort, burning) most prominent in an area of tumor growth; this syndrome appears most frequent in patients with Hodgkin's disease and lymphomas. Clinical implications, including the special difficulties in treating alcoholic patients are discussed, and a general proposal made: that decreased exposure to alcohol would be the most effective method to achieve a significant decrease in cancer deaths. (54 references)

0002 SOME BIOCHEMICAL ASPECTS OF CADMIUM TOXICOLOGY. (Eng.) Buell, G. (Dep. Health, State Calif., Berkeley). *J. Occup. Med.* 17(3):189-195; 1975.

Data on cadmium concentrations in the air and lungs of exposed populations of 36 U. S. cities, on cadmium concentrations in fresh waters, and on atmospheric cadmium concentrations in various cadmium-using industrial operations are reviewed. Cases of acute and chronic occupational cadmium poisoning are documented. The most characteristic manifestation of cadmium poisoning is the appearance in the urine of a low-molecular weight protein that apparently consists largely of light chain immunoglobulins. Most exposed workers have impaired renal function due to tubular damage. Chronic exposure to atmospheric cadmium results in emphysema, which usually appears after 20 yr but may occur after only two yr of exposure. Although a cause and effect relationship has not been established, cadmium exposure has been linked to prostatic cancer. Of 248 workers exposed to cadmium oxide dust for a minimum of one yr, four developed prostatic cancer where 0.58 cases were expected. In animals, cadmium has a site-specific teratogenic effect; i.v. injection of cadmium sulfate in hamsters results in a high incidence of facial and other malformations including exencephaly and anophthalmia. Evidence that cadmium binds to phosphates or the bases of RNA suggests that it may induce genetic alterations. Since some of the toxic manifestations of cadmium can be prevented by zinc, the zinc-cadmium ratio may be important in the biochemistry and physiology of cadmium. The monitoring of exposed workers is suggested to be sure that urinary cadmium concentrations remain below 15 µg/g creatine, the level at which the probability of renal damage is low. A downward revision of the Threshold Limit Value for cadmium is imperative. (106 references)

0003 FOOD. (Eng.) Sloman, K. G. (Gen. Foods Tech. Cent., White Plains, N.Y.); Foltz, A. K.; Yeranslan, J. A. *Anal. Chem.* 47(5):56R-84R; 1975.

An exhaustive survey of advances, innovations, and improvements in food analysis is presented. Determination of additives employs both old and new techniques, including colorimetric detection, silica gel column, gas, thin layer, gel, and paper chromatography, and fluorimetry. Detection of the adulteration, contamination, and decomposition of various foods is discussed; the reporting of contaminants increases in proportion to the sensitivity of analytical methods. Major attention is given to the formation of nitrosamines and the presence of polycyclic aromatic hydrocarbons, mycotoxins, and diethylstilbestrol in foods. Research on the determination of carbohydrates in foods is surveyed; and the stability, chemical structure, degradation, and methods of analysis for anthocyanins in foods is reviewed. A number of analytical techniques for the analysis of enzymes in foods are noted. Numerous methods employed isolation and analysis of fats, oils, and fatty acids are also noted. In reviewing the instruments and methodologies for the isolation, fractionation, and identification of flavors and volatile compounds, special use is noted of gas chromatography. Reports on recent information on the basic composition of foods, and new techniques used in their characterization, are recorded for a wide variety of foodstuffs. Analysis of traces of inorganic constituents in food materials proceeds with much attention devoted to necessary and other heavy meatals. The determination of moisture by a variety of methods is reported; analysis of organic acids features carboxylic acids and phenolic acids most extensively. Nitrogen analysis *via* improvements in a variety of techniques is reported, while advances in vitamin determinations in food tend to be more instrumentally dependent. Additional miscellaneous reports include surveys on determination and detection of emulsifiers and stabilizers, on the chemistry of amino acid-reducing sugar reactions, and on the utilization of automated wet-chemical analyzers. (1,030 references)

0004 ASSESSMENT OF HUMAN EXPOSURE AND RESPONSE TO *N*-NITROSO COMPOUNDS: A NEW VIEW ON THE ETIOLOGY OF DIGESTIVE TRACT CANCERS. (Eng.) Weisburger, J. H. (Naylor Dana Inst. Dis. Prev., Valhalla, N.Y.); Ranieri, R. *Toxicol. Appl. Pharmacol.* 31(3):369-374; 1975.

Over 100 substances of the nitrosamine class are carcinogenic in animal models, although their role in human carcinogenesis has not been demonstrated. Some, like *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, readily induce gastric cancer in a number of mammalian species, including non-rodents. These chemicals are active after nonenzymic hydrolytic activation, and are active wherever inserted (e.g., colon, stomach) or applied (e.g., skin). Because of rapid detoxification, they are not active at sites far from the point of application, and even limited exposure p.o. will induce gastric cancer, or colonic cancer when introduced rectally. The epidemiology of human gastric cancer correlates with these properties. Individuals moving from high-risk to low-risk areas maintain their propensity for developing gastric cancer, if they have lived in the high-risk area for the first 10-15 yr of their lives. Gastric cancer in the U.S. has declined appreciably in the last 40 yr, but the incidence is still higher in the lower socioeconomic groups than in middle or upper classes. It is proposed that this decrease results from improvement of diet, and from the use of refrigeration as a means of food preservation. Nitrate in various types of food left at room temperature becomes reduced to nitrite, which may then react with naturally occurring substances such as methylguanidine to form carcinogenic nitroso-derivatives. It is concluded that gastric, and perhaps esophageal and hepatic cancers may be due to formation of nitrosamides and nitrosamines derived from microbiologic conversion of nitrate to nitrite in food stored at room temperature. (26 references)

0005 NITROSAMINES AND CANCER. (Eng.) Anonymous. *Nutr. Rev.* 33(1):19-20, 1975.

That nitrosamines are produced *in vitro via* the reaction of secondary and tertiary amines with nitrites leads to a discussion of possible environmental sources of such carcinogens. Investigation of cured, nitrite-containing food products led to the discovery of small but varying quantities of nitro samines in such foods, and a signigicant contamination of one brand of sausage. It is further established that spices such as black pepper and paprika react with nitrites to form nitrosamines. Effects of the *in vivo* formation of nitrosamines as a result of ingesting nitrites and amines, is studied in rats. It is noted that rats fed heptamethyleneimine hydrochloride plus sodium nitrate produced fatal tumors, primarily pulmonary and esophageal cancers. Rats fed two dose levels of aminopyrine plus sodium nitrite developed mainly hepatic tumors, whereas no deaths were attributed to the ingestion of the individual compounds alone. The experiments are claimed to suggest that the interaction of secondary and tertiary amines with nitrites in the stomach may represent an important facet of the etiology of human cancers. Furthermore, it is noted that nitrosamines present in tobacco smoke condensates may be contributory to nitrate ingestion and the effect of dietary carcinogens may be modified by the level of dietary protein and lipotropic factors. No data is yet available on the contribution of protein, mineral, and vitamin intake to *in vivo* carcinogenesis. (8 references)

0006 VINYL CHLORIDE: A REPORT OF A EUROPEAN ASSESSMENT. (Eng.) Van Esch, G. J. (Natl. Inst. Public Health, Bilthoven, Netherlands); Van Logten, M. J. *Toxicology* 4(1):1-4; 1975.

A meeting of European toxicologists was held to assess the available toxicological and migration data on vinyl chloride, with special reference to its carcinogenic potential. Because of indications that vinyl chloride induces angiosarcomas in exposed workers, it is considered that 50 ppm vinyl chloride in inspired air is too high to adopt as a Threshold Limit Value. Present industrial exposure levels should be reduced as far as possible, and efforts should be made to eliminate the hazard to operatives of exposure to high vinyl chloride levels during the cleaning of polymerization vessels. Data on the migration of vinyl chloride from polyvinyl chloride (PVC) indicate that PVC used for food and drink containers and wrappings should contain less than 20 ppm vinyl chloride. This is necessary to maintain very low levels of contamination of food. The final solution of the vinyl chloride problem will depend on the evaluation of further data, including (a) epidemiologic studies on vinyl chloride-linked diseases in man, (b) levels of industrial and environmental exposure, (c) effects of p.o. vinyl chloride in animals, (d) investigations to determine if induction of liver tumors by vinyl chloride is preceded by liver dysfunction and cirrhosis, (e) metabolism of vinyl chloride, (f) percutaneous migration of vinyl chloride, (g) estimated daily dietary intake of vinyl chloride in children and adults, (h) interaction of vinyl chloride with food and drink components, (i) vinyl chloride levels in PVC products, and (j) vinyl chloride levels in potable water and PVC tubing. (9 references)

0007 THE ONCOGENE HYPOTHESIS AND THE SEARCH FOR HUMAN CANCER VIRUSES. (Eng.) Pringle, C. R. (Inst. Virol., Glasgow, Scotland). *Scott. Med. J.* 20(2):61-67: 1975.

A review of literature pertaining to the oncogene hypothesis centers around a discussion of RNA tumor viruses. Whereas the importance of DNA viruses as oncogenic agents under natural conditions is controversial, the common property of all oncogenic viruses is the multiplication in the cell nucleus and integration into the nuclear DNA; cytoplasmic RNA viruses have no oncogenic potential. Three morphological forms of RNA tumor virus particles are distinguished by electron microscopy and designated A-, B-, and C-type particles. A study of the virus components re-

veals two major species having sedimentation coefficients of 60-70S and 4-5S; the former represents the viral genome, and the latter functions as links or primers. In common with other enveloped viruses, several enzymatic activities are associated with RNA tumor virus particles; this includes reverse transcriptionase activity. Evidence of viral genomeintegration into host DNA is obtained from nucleic acid annealing experiments and genetic studies. The susceptibility of cells to infection by RNA tumor viruses is found to be controlled by genetic factors in the host genome, with loci affecting the inducibility and expression of endogenous viral genetic information. The oncogene hypothesis of tumors proposing the integration of a virogene in the DNA of all cells, which may include oncogenic information, is presented. An alternate hypothesis, introducing the concept of the protovirus, is also discussed. It is noted that several avian, murine, and feline sarcoma-inducing viruses have been isolated, although no human C-type viruses are yet unequivocally identified or isolated. However, attempts to detect putative viral genomes and gene products in human cells are more successful, and a variety of probes are being used in the search for cryptic viruses in human tumor tissue. (11 references)

0008 CLINICAL ASPECTS OF INFECTION WITH THE EPSTEIN-BARR VIRUS. (Eng.) Sutton, R. N. P. (King's Coll. Hosp. Med. Sch., London, England). *J. R. Coll. Physicians Lond.* 9(2):120-128; 1975.

The association of the Epstein-Barr virus (EBV) with Burkitt's lymphoma, nasopharyngeal carcinoma, infectious mononucleosis, and various other conditions are discussed. Whereas the EBV is a very closely cell-associated virus, information on the association of the virus with disease depends mainly upon serological procedures involving distinct antigen-antibody systems. Despite the wide distribution of EBV, the virological tests are of potential practical use in the elucidation of unusual manifestations of disease in early infancy. Infection with EBV generally occurs in early childhood *via* an asymptomatic serologic conversion, with little further EBV activity until early adult life. During active infectious mononucleosis, total IgM, IgA, IgG, and IgE immunoglobulins are elevated, and oropharyngeal excretion of EBV persists for many weeks; anti-nuclear, anti-IgC, anti-i, anti-smooth muscle and anti-lymphocyte activity also occur. A discussion of unusual manifestations of EBV infection notes neurological features involving cerebrospinal fluid, factors of iatrogenic infection, and involvement of EBV in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, plus other malignant diseases. High levels of EBV antibody are found in sarcoidosis, and are claimed in conflicting reports of systemic lupus erythematosus. Apart from normal methods of infection, EBV is found transmitted by blood transfusion and reactivated following immunosuppression. It is suggested that infection with EBV may result in a wider spectrum of illness than previously suspected, and that EBV is potentially susceptible to antiviral drugs. (90 references)

0009 B CELL ACTIVATION. (Eng.) Waldmann, H. (Dep. Pathol., Cambridge, England); Munro, A. *Transplant. Rev.* 23:212-222; 1975.

A discussion of the two 'signals' considered essential for B cell activation is presented, and the potential role of T cells is reviewed. Models proposed to account for the mechanism of induction of B lymphocytes define at least three discrete processes involved in the development of an 'immune' state: division of B cells, production of 'memory daughter' cells, and maturation towards antibody production. Evidence suggests that B cells producing antibodies of different classes have different inductive requirements. The current working hypothesis assumes two signals, the amount, duration, and time sequence of which may be of critical importance. Stage I involves the aggregation of surface immunoglobulin (Ig); the hypothesis predicts that the detectable effects are either the production of unresponsiveness in the absence of second signal, or the induction of an antibody response in the presence of second signal. Experimental investigations using TNP-BSA, DNP-HSA, TNP-LPS, and Fab'_2 fragments of specific antibody support the suggestion. Whereas active suppressor mechanisms may be required in some situations, further evidence to support the importance of a synergy between multivalent antigen and nonspecific signals comes from experiments in which a potentially tolerogenic stimulus is abrogated by a nonspecific T cell activity. Two types of experiments argue against the suggestion that aggregation of Ig represents the first signal; these include data suggesting that monovalent haptens can provide the relevant first signal, and the independent observation that TNP conjugates, coupled at critical concentrations to presumed inert particles, could act as thymus-independent antigens for an IgM response *in vitro*. A variety of sources of second signal are suggested, plus evidence that macrophages can produce a nonspecific factor capable of facilitating a B cell response *in vitro*. Implicit in the hypothesis is the factor that thymus-independent antigens can, in the absence of T cells, either generate or mimic the second signal. No restriction of genetic compatibility of cells is yet established. It is suggested that interaction of T cell-specific factor with I region coded products on B cells may enchance signal I by stabilizing the interaction of antigen/T cell receptor complexes on the cell membrane. (38 references)

0010 CYCLIC NUCLEOTIDES AS INTRACELLULAR MEDIATORS OF B CELL ACTIVATION. (Eng.) Watson, J. (Salk Inst., San Diego, Calif.). *Transplant. Rev.* 23:223-249; 1975.

The possible involvement of the cyclic nucleotides adenosine 3':5' monophosphate (cAMP) and guanosine 3':5' cyclic monophosphate (cGMP) as the intracellular mediators of the expression of B cells is discussed. The general concept of intracellular mediators in B cell activation, and cell development along two possible pathways (inductive and paralytic) are noted. Studies indicate that B cell mitogens exert their effects at the cell surface and that

interaction with select membrane components is necessary; both specific and nonspecific membrane interactions may be acknowledged. Many changes in cellular metabolism are detected following the addition of mitogenic agents to lymphoid cells; particular attention is given to cGMP and cAMP levels. Subcellular distribution of adenylate and guanylate cyclases is studied, as is the possible interaction of B cell mitogens with such cyclases. Evidence indicates that B cell mitogens bind to membranes, causing some change that indirectly leads to the activation of guanylate cyclases. Further studies are done on the activation of guanylate cyclases by inorganic ions, with particular attention to calcium. Investigations of the nature of the signal indicate that the cells respond not to the absolute level of cGMP, but to the intracellular ratio of cAMP to cGMP. The effects of such extracellular cyclic nucleotides or cyclic nucleotide-elevating agents on the induction of primary immune responses are discussed, plus the inactivation of B cells by cAMP. A lengthy consideration of cAMP and cGMP as intracellular mediators of B cell pathways is presented. Whereas little is known of how adenylate and guanylate cyclases are linked to cell surface receptors, it is suggested that an intracellular ratio between the two governs the response of a cell, as mediated by the delivery of T cell signals. Although the biochemical mechanisms are not resolved, it is suggested that activators of B cells effect a further step in their developmental sequence, and that cyclic nucleotides and inorganic ions may be intracellular mediators of developmental sequences in most eukaryotic cells. (108 references)

0011 IS THERE A STIMULATOR CELL FOR B-LYMPHOCYTES? (Eng.) Talmage, D. W. (Univ. Colorado Sch. Med., Denver); Thomas, D. W. *Transplant. Rev.* 23:202-212; 1975.

Factors capable of promoting the activation and proliferation of B-lymphocytes are reviewed, and a stimulator cell model is proposed. The relationship between B-lymphocyte proliferation and immunoglobulin (Ig) synthesis, as studied in bone marrow and fetal liver transplants, suggests a close correlation between antibody-producing cell proliferation and antibody production. Agents blocking DNA synthesis or mitosis effect a dramatic reduction in antibody production, as do various mitogenic agents; the observations suggest a linkage between Ig synthesis and proliferation in mixed cell populations. In addition, discontinuous antibody production is reported, and related to the expression of genetic information for Ig synthesis. Observations on the activation of B-lymphocytes by antigen and mitogens are summarized, and proposals explaining B-cell, activation by antigen fall in two general categories, direct and indirect. The direct activation of B-cells by antigen is generally attributed to a particular conformation or pattern of determinants allowing for the cross-linking of surface receptors, whereas proposals for indirect B-cell activation by antigen generally assign the antigen a role of delivering some other extracellular mitogenic agent. In considering the concept of a stimulating cell, it is proposed that it may be necessary for the activation of both T and B lymphocytes in the presence of antigen. A model presented for the collaboration between the stimulator cell and T and B-lymphocytes allows antigen activation of the B-cell by three routes: direct, indirect *via* activation of the stimulator cell, and indirect *via* activation of T cell and stimulator cell. It is postulated that the T-cell factors operate *in vivo* against a background of suppression, and hence are short range; thus, the location of all cells within a single cell clump presents a distinct advantage. Several predictions, based on the model, are presented. (56 references)

0012 CELL MEMBRANES AND DISEASE: GENES, VIRUSES, HORMONES AND THE IMMUNE RESPONSE--AN INTRODUCTION. (Eng.) Dmochowski, L. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.); Bowen, J. M. *Am. J. Clin. Pathol.* 63(5):619-628; 1975.

A description of the morphology, chemistry, and interactions of cell membranes is discussed. Electron microscopy, X-ray diffraction, spectroscopy, immunoelectron microscopy, and various biological methods yield information on the structure and function of cell membranes. Membrane abnormalities involving enzymes of lipid metabolism are acknowledged and hormonal effects are ascribed to changes in cellular function. The presence of a three-component system in plasma membranes, consisting of the receptor, effector, and transducer, is also noted. The interaction of drugs, vitamins, and hormones is studied by a variety of physical methods. Viruses have only recently been recognized as tools in cell membrane research. Viral envelopes and cellular membranes have many morphologic and chemical features in common. Immunologic changes appear genetically best defined as plasma membrane alterations of the neoplastic state, because the immune response involves the plasma membrane of cells as carrier of specific groups of surface topography, various stages of differentiation, etc. Research on immunogenetics of cell surfaces centers on the relation of cell surface antigens to the transplantability of tissues and organs. Isolation and purification of glycoproteins from cell membranes of human tumors are described. Changes in the architecture of cell surface are observed to occur in many chemically induced tumors, with two types of membrane changes involving glycoproteins and glycolipids noted: enhanced agglutinability and blocked syntheses. Cyclic adenosine monophosphate is implicated in the restoration of normal properties to transformed cells, affecting cell morphology, growth rate, and density. Morphologic expression of cell surface transformation and associated surface changes are attributed to the expression of different sarcoma genes. The accumulation of acid mucopolysaccharides is also assigned a role in the modified behavior, which includes reduced cellular adhesion, the ability to grow in solid agar, and diminished responses to a variety of external regulatory stimuli. (32 references)

0013 THE GENETICS OF CHILDHOOD CANCER. (Eng.) Knudson, A. G., Jr. (Univ. Texas Health Sci. Cent., Houston). *Cancer* 35(3):1022-1026; 1975.

Retinoblastoma should not be considered as the excep-

tional childhood cancer that shows dominant inheritance, but rather as the typical childhood cancer that embraces a prezygotic and postzygotic subgroup. For retinoblastoma, the prezygotic group contains about 40% of the cases. Estimates of 38% and 22% have been made for Wilm's tumor and neuroblastoma, resp., while no estimates can yet be made for the other embryonal tumors. The postzygotic cases are conceived as involving mutation as a first step too, but with the mutation in somatic rather than germinal cells. In both prezygotic and postzygotic cases a second event, possibly mutational, occurs before the cancer is initiated. The embryonal cancers are all visualized as genetic diseases, and their frequencies are limited by gene mutability. This mutability may be increased by such environmental agents as radiation, chemicals, and viruses. Prezygotic cases are of particular concern since they are predisposed to other primary tumors in the same organ(s) and may be predisposed to primary tumors in other tissues. A diagnostic test to identify prezygotic cases among those with a single primary tumor and a negative family history is needed. Because of the high concordance (25%) in monozygotic twins and the numerous reports of familial cases, leukemia and lymphoma may also constitute a prezygotic group. (17 references)

0014 EPITHELIAL NEOPLASIA -- A HOMEOSTATIC DISORDER. (Eng.) Smithers, D. (No affiliation given). *Laryngoscope* 85(2):279-286, 1975.

General aspects of oncology are reviewed with emphasis on epithelial neoplasia. It is proposed that epithelial tumors are similar tissue reactions occurring in response to a great variety of causes, and that they are a result of the failure of a normal growth control system which allows the establishment of new levels of proliferation with a tendency to progression. Cancerous reactions express a degree of disorganization which not only varies from one reaction to another but also from time to time in any one reaction. Despite the great number of possible cancerous reactions these changes are quite rare in most parts of the body, the majority being concentrated in comparatively few tissues. The tissues most liable to neoplastic reactions have certain significant things in common: they are either those surfaces at which demands are often made for repair of damage done by the environment or those organs subjected to cyclical and often to thwarted demands for function. With many forms of demand, a great variation in susceptibility, and differing reasons for failure of control, the causes of epithelial neoplasia are numerous and complex. When the stresses are applied over a wide area, multiple tumors may appear; on the other hand, in some cases it is the susceptibility which is widespread, giving rise to multiple tumors on little provocation. It is maintained that epithelial tumors do not suddenly spring to life by malignant transformation in single cells, but develop slowly and in stages, often appearing multicentrically over a region of affected tissue and commonly progressing from a conditional to a more nearly independent state, as disorder creates conditions favoring further disorder. (14 references)

0015 EPIDEMIOLOGY: PROBLEMS IN THE STUDY OF CANCERS OF LOW INCIDENCE AND THE NEED FOR COLLABORATION. (Eng.) Miller, A. B. (Natl. Cancer Inst. Canada, Toronto). *J. Natl. Cancer Inst.* 54(2): 299-301; 1975.

Cancers for which no clear hypotheses currently exist include: carcinomas of the kidney, carcinomas of some parts of the digestive system, non-Hodgkin's lymphomas, tumors of the nervous system and many sarcomas. The problem of rare cancers is reviewed in relation to how the epidemiologist obtains an appropriate hypothesis and how he secures support for an exploratory study which may be criticized for lack of a clear hypothesis to test. Disadvantages of the case-control or cohort approaches are noted, including the tendency to change design or the nature of the data sources as time passes and the inability to dissociate the contribution of the various factors which together cause the cancer. In developing hypotheses for evaluation in man or in laboratory animals, the epidemiologist has an obligation to take into account all available knowledge. Solutions to this problem include the multidisciplinary approach being fostered by the International Agency for Research on Cancer, multidisciplinary workshops to consider more focused attacks, and also multicenter-controlled investigations. With the use of the resources of several centers and with a carefully thought-out common design and instrument, studies of a rare condition will result in more rapid answers than are possible from one institution. This approach will be more expensive for the granting agency but will be more "cost-effective" than alternative approaches. (15 references)

0016 LARGE-BOWEL CANCER: AN EPIDEMIOLOGIC JIGSAW PUZZLE. (Eng.) Burkitt, D. P. (Med. Res. Counc., London, England). *J. Natl. Cancer Inst.* 54(1):3-6; 1975.

Available information on large bowel cancer is reviewed in an effort to formulate a hypothesis of its etiology. This information includes such facts as the close association of the prevalence of tumors of the large bowel with economic development and with urban life; the prevalence increases on migration from a less-westernized to a more-westernized country. Most of the benign and malignant tumors occur in the areas of the bowel where the feces tend to stagnate, suggesting a relationship between tumor induction and prolonged contact between the feces and the bowel mucosa. The feces from populations with a high risk of bowel cancer contain more anaerobes than do the feces from people of low-risk areas. This suggests that fecal bacteria play an important role in human bowel cancer and that the bacterial degradation of the bile acid cholate to potentially carcinogenic deoxycholate may be significant. The addition to diet of fiber in the form of bran inhibits the bacterial degradation of cholate. Populations with relatively short intestinal transit times and with large, soft stools containing low concentrations of degraded bile acids have a low prevalence of large bowel tumors; communities with prolonged transit times, small

firm stools, and high fecal concentrations of degraded bile acids have a higher prevalence of large-bowel tumors. Vegetarians in North America tend to have a lower risk of developing bowel cancer than do non-vegetarians. This evidence suggests that large bowel tumors are related to some factors characteristic of modern Western society, which slow down intestinal transit times, produce small firm stools, and alter the fecal bacterial flora. One such factor could be the lower consumption of dietary fiber and the higher consumption of dietary fats. It is suggested that fiber be restored to the diet in the form of whole-meal bread and bran. (26 references)

0017 OCCUPATIONAL SKIN CANCER: A REVIEW. (Eng.) Emmett, E. A. (Univ. Cincinnati Coll. Med., Ohio). *J. Occup. Med.* 17(1):44-49; 1975.

The major causes of occupational skin cancer are UV radiation and polycyclic aromatic hydrocarbons. Lesser causes include arsenic, ionizing radiation, and miscellaneous factors such as heat and trauma. The histologic types of skin cancer associated with excessive sun exposure are solar keratoses, basal cell epitheliomas, squamous cell carcinomas, keratocanthomas, and malignant melanomas. No studies appear to have been done relating skin cancers with industrial UV sources. Prevention of UV carcinogenesis depends on minimum exposure of abnormally susceptible or predisposed persons, protective clothing and screening of industrial UV sources, avoidance of simultaneous exposure to UV and chemicals that augment UV carcinogenesis, appropriate sunscreens, and periodic review for early detection of skin lesions. Animal studies show that the susceptibility of the skin to polycyclic aromatic hydrocarbons such as benzo(a)-pyrene is influenced by the presence of such accelerators as long-chain aliphatic and aromatic hydrocarbons. In man, the role of accelerators is seen when latent periods for various carcinogenic exposures are compared. Exposure to coal tars and pitch (high in polycyclic carcinogens) results in tumors within 20-24 yr. Spindle oil exposure (low in carcinogens, low in accelerators) results in tumors after 50-54 yr, whereas exposure to slack wax (low in carcinogens, high in accelerators) can result in cancer after a 20-yr latent period. Measures for minimizing the skin cancer hazard include the substitution of a noncarcinogenic for a carcinogenic material where possible, compulsory showering, protective clothing, closed system operations, avoidance of simultaneous or closely apposed exposure to UV and periodic review. Skin cancers induced by arsenic are squamous cell carcinomas, basal cell epitheliomas and intraepidermal carcinomas. Tumors associated with ionizing radiation are predominantly squamous cell carcinomas, followed by basal cell epitheliomas and, more rarely, malignant melanomas. The latent period for radiation-induced skin tumors varies inversely with the dose and averages 25-30 yr. The skin cancer may be induced by dose-equivalents of about 3000 rem. Examples of miscellaneous factors contributing to the genesis of skin cancer are squamous cell carcinomas in burn scars and around chronic draining sinuses. Prevention of such tumors consists of prompt treatment of the underlying cause. (82 references)

0018 ENVIRONMENTAL FACTORS IN THE ORIGIN OF CANCER AND ESTIMATION OF THE POSSIBLE HAZARD TO MAN. (Eng.) Jones, H. B. (Lawrence Berkeley Lab., Univ. Calif., Berkeley); Grendon, A. *Food Cosmet. Toxicol.* 13(2):251-268; 1975.

The dose-response relationship to cancers, and several examples of the estimation of cancer risk are presented. The hydrocarbons and ionizing radiation show similar dose-effect relationships with respect to carcinogenesis with the probability of inducing a tumor per unit dose apparently a constant for any given carcinogenic agent. Furthermore the relationship between dose and time occurs similarly in all species tested, and is observed as an inverse cube-root relationship between latent period and dose. A model is presented, affording a biological basis for the observed phenomenon that latent period is inversely proportional to a fractional power of the dose of various carcinogens, with a value close to 0.33 for the exponent. A review of the data on the latent period for the appearance of leukemia in the Japanese survivors of atomic bombings, plus a study on radium-induced bone cancer latency, support and extend the hypothesis. The latter serves to advance the concept that a practical threshold to a carcinogenic effect exists and that the expected lifespan may be exceeded by the time necessary for low concentrations of altered cells to develop into cancers. A study on the family of dialkylnitrosamines shows similar dose-response relationships to those aforementioned. Conclusions drawn from experimental carcinogenesis in laboratory rats indicate that the risk of cancer induction varies with the degree of exposure, and that the relationship between latent period and dose is as similar for man and other animal species. The dose-response relation is also studied *via* the induction of lung-tumor modules in mice by urethane anesthesia, and again concludes a dose-dependence in the number of tumor foci induced. An extensive mathematical analysis is presented for the estimated cancer risk from diethylstilbestrol (DES), and concludes no hazard from DES residues in treated beef, either in consumers or their offspring. A theory of the stages in the development of cancer is presented, and a central inference may be made: the critical process in carcinogenesis is a failure of organ controls over the proliferative tendency of clone or clones of cells that form a cancer. Whereas urethane induction of lung tumors appears the sole exception to the hypothesis of tissue-control factors, a corrolary is also presented, suggesting a dependency of the rate of interaction on the proximity of the affected cells. (33 references)

0019 SHOULD WE REGARD HODGKIN'S DISEASE AS INFECTIOUS? (Eng.) Anonymous. *Br. Med. J.* 1(5954):351-352; 1975.

Epidemiological aspects of several outbreaks of Hodgkin's disease are reviewed in an attempt to determine if the disease might be infectious. In one such outbreak in a school, four students who had attended one school at the same time developed Hodgkin's disease; an additional 31 cases (with direct contact with the original four or through a

single intermediary) were identified. This outbreak extended over a period of 20 yr. A similar type of outbreak with five cases occurring over a period of 11 yr in a small township is also discussed. In a study of Hodgkin's disease in the family, there was about a 3-fold increase in incidence among first-degree blood relatives. The time interval between diagnoses was shorter when the two individuals lived in the same household than when they lived apart. These observations favor an environmental rather than a genetic etiology. In the northeastern United States and most northern European countries, there is an early peak between 15 and 35 yr of age and a later peak over the age of 50. This has led to the suggestions that Hodgkin's disease is an infectious condition in the young and a neoplasm in the old or that older people acquire the disease from their children. The early peak in incidence is lacking in Japan but does appear in Japanese living in the United States. This shift in the American-Japanese curve towards that of white Americans has been interpreted as being consistent with an environmental influence affecting all ages (rather than with an infective agent affecting only young adults). On current evidence, therefore, it is premature to conclude that infectious transmission of Hodgkin's disease occurs in the school or family. The postulated increase in incidence in these situations is only of the order of 2- to 8-fold. In practical terms, this represents a negligible hazard in a disease with an overall incidence of 4/100,000/yr. (17 references)

0020 DYNAMIC CHANGES ON THE SURFACES OF NORMAL AND TRANSFORMED CELLS AND THEIR RELATIONSHIP TO THE ENTRY OF PHARMACOLOGICALLY ACTIVE PROTEINS. (Eng.) Nicolson, G. L. (Salk Inst. Biol. Stud., San Diego, Calif.). *Am. J. Clin. Pathol.* 63(5):677-684; 1975.

The relationship between neoplastic membrane dynamics and the mechanism by which specific toxins preferentially kill tumor cells is discussed. The fluid mosaic membrane model considers the thermodynamic principles governing the organization of biomembranes with structures maximizing the lowest possible free energy environments for lipids, proteins, and saccharides. In view of this hydrophilic and hydrophobic interactions, lateral mobility of membrane components, and surface receptors are discussed. Mention is given of the various cell-surface properties altered after neoplastic transformation, and a discussion of the mechanism of preferential killing of tumor cells by toxins is presented. Special mention is given to the investigation of the mechanism of Ricinus communis II agglutin (RCA_{II}) toxin killing and the differences between normal and tumor cells. An electron-dense ferritin conjugate of RCA_{II} is employed in investigating the possible entry of toxin into cells, using especially 3T3 and SV3T3 cells; it indicates that RCA II apparently binds to and enters both normal and transformed cells by similar mechanisms *via* similar numbers of cell-surface receptors. However, the dynamics of certain receptors on the surfaces of normal and transformed cells appear different. Differences in receptor mobility, aggregation, endocytosis, etc. may be critical factors in drug, toxin, lectin, antibody and cell interactions, leading to differential tumor cell death without appreciable host cell toxicity. (48 references)

0021 THE GENERATION OF CHEMICALLY REACTIVE METABOLITES: EPOXIDES AS REACTIVE INTERMEDIATES IN AROMATIC HYDROCARBON METABOLISM. (Eng.) Sims, P. (R. Cancer Hosp., London, England). *Biochem. Soc. Trans.* 3(1):59-62; 1975. (21 references)

0022 ALKYLATING INTERMEDIATES IN NITROSAMINE METABOLISM. (Eng.) Magee, P. N. (Middlesex Hosp. Med. Sch., London, England); Nicoll, J. W.; Pegg, A. E.; Swann, P. F. *Biochem. Soc. Trans.* 3(1):62-65; 1975. (32 references)

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0030 TOBACCO AND PUBLIC HEALTH: FIRST ATTEMPT AT DETOXIFICATION. (Fre.) Chicou, F.-J. (Section Recherche en Actions de Sante Publique INSERM 44, chemin de Ronde, B. P. 34, F 78110 Le Vesinet, France). *Bull. Cancer (Paris)* 62(1):103-119; 1975. (No references)

0031 LEUKEMIA IN CHILDHOOD: INTRODUCTION AND ETIOLOGY. (Eng.) Hartmann, J. R. (Child. Orthop. Med. Cent., Seattle, Wash.). *Cancer* 35(3/Suppl.):996-999; 1975. (23 references)

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0033 OCCUPATIONAL BLADDER CANCER. (Eng.) Parkes, H. G. (British Rubber Manuf. Assoc. Ltd., Birmingham, England). *Practitioner* 214(1297):80-86; 1975. (27 references)

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0050 PARAKERATOSIS MIBELLI AND SKIN CARCINOMA; A CRITICAL REVIEW. (Eng.) Goerttler, E. A. (Dept. Dermatol. Univ. Heidelberg, West Germany); Jung, E. G. *Humangenetik* 26(4):291-296; 1975. (24 references)

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0057 PRIMARY HEPATOMA IN JAPAN. (Ger.) Hattori, N. (Natl. Cancer Cent. Hosp. Tokyo, Japan); Okazaki, N.; Ohno, T.; Mukojima, T.; Ohkura, H.; Araki, E.; Kitaoka, H.; Hasegawa, H. *Leber Magen Darm* 5(2):58-61; 1975. (8 references)

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0059 INTERPRETING THE RATE OF SURVIVAL IN CARCINOMA. (Eng.) Smith, C. (George Washington Univ. Med. Cent., Wash., D. C.). *Can. J. Surg.* 18(2):129-132; 1975. (11 references)

0060 DETECTION OF CARCINOGENS AS MUTAGENS: BACTERIAL TESTER STRAINS WITH R FACTOR PLASMIDS. (Eng.) McCann, J. (Biochem. Dep., Univ. California, Berkeley); Spingarn, N. E.; Kobori, J.; Ames, B. N. *Proc. Natl. Acad. Sci. USA* 72(3):979-983, 1975.

The detection of carcinogenic agents as mutagens in bacterial test strains with a R factor plasmid was investigated. R factor transfer was carried out using R-factor-containing donor strains and recipient strains diluted in nutrient broth. Ampicillin-resistant recipients were selected on petri plates containing ampicillin and required supplements (histidine and biotin). Donor strains SL1156 (*trpD1*/R Utretcht), SL1127 (*pur pro*/R46) and TA2000 (*purF145*/KM101) were produced by transforming pKM101 from *his-G46*/pKM101 to *purF145*. R factor and tester strains were maintained at -80 C after freezing nutrient broth with dimethyl-sulfoxide. The R factor plasmid in bacterial strains developed two new strains (TA-100 and TA98) which significantly increased the number of carcinogens as mutagens. Aflatoxin B, sterigmatocystin benzo[*a*]pyrene, 7,12-dimethylbenzanthracene were carcinogenic mutants more readily detected with the two new strains. *HisG46* containing pKM101 and TA100 (TA135 with pKM101) was most sensitive to methyl methanosulfonate-induced reversion and pSC101 was found to be inactive. Mutagens found in this study seemingly worked more effectively due to an error-prone recombinational repair of DNA. Further investigation into the mechanism of the R plasmid enhancement of mutagenesis is indicated.

0061 CORRELATION BETWEEN BALANCE OF SPECIFIC CHROMOSOMES AND EXPRESSION OF MALIGNANCY IN HAMSTER CELLS. (Eng.) Benedict, W. F. (Child. Hosp. Los Angeles, Calif.); Rucker, N.; Mark, C.; Kouri, R. E. *J. Natl. Cancer Inst.* 54(1):157-162; 1975.

To determine whether a specific chromosome imbalance could be correlated with the expression of malignancy in transformed cells, the chromosome patterns in fetal hamster (HF) cells transformed by 1-β-D-arabinofuranosylcytosine (ara-C) and dimethylnitrosamine (DMN) were studied. Secondary HF cells were incubated *in vitro* with 0.1 μM ara-C or DMN for 24 hr. Four ara-C- and one DMN-transformed line were thus established. The transformed cells (2×10^6) were injected s.c. into newborn hamsters, in which they produced tumors. Two of the fibrosarcomas produced by the ara-C-transformed cells were cloned and their chromosome patterns studied, along with their ability to produce tumors *in vivo*. Specific chromosome changes appeared both in the ara-C-transformed cells and in the fibrosarcomas arising from them: metaphases contained 47 chromosomes and had a normal diploid complement except for one extra each of chromosomes 4, 5_7 and 5_{12}. Clones with high and low malignant potentials were derived from the two fibrosarcomas produced by the ara-C-transformed cell line. The expression of malignancy in these clones was associated with an excess of 5_7 chromosomes over 7_3 chromosomes, clones with a 7_3-5_7 chromosome ratio of one or more having low tumorigenicity. The variant clones of low malignancy appeared unstable in culture, becoming highly tumorigenic after several passages. There was some evidence that genetic information for the expression of malignancy was present on the long arm of the 5_7 chromosome.

0062 UNLIMITED DIVISION POTENTIAL OF PRECANCEROUS MOUSE MAMMARY CELLS AFTER SPONTANEOUS OR CARCINOGEN-INDUCED TRANSFORMATION. (Eng.) Daniel, C. W. (Div. Nat. Sci., Univ. California, Santa Cruz); Aidells, B. D.; Medina, D.; Faulkin, L. J., Jr. *Fed. Proc.* 34(1):64-67; 1975.

The correlation between cell transformation and unlimited growth potential was studied to determine whether this relationship is causal or merely casual. In particular, the growth potential of precancerous mouse mammary cells (appearing as nodules in the mammary glands of old, multiparous mice of certain strains) was studied after spontaneous and carcinogen-induced transformation. Serial transplantation of normal mouse mammary tissues in young, isogenic hosts had previously been shown to result in a progressive loss of division potential, the transplant line eventually being lost. This is interpreted as an expression of senescence at the cell and tissue level, and it inevitably occurs despite optimal growth conditions. An indefinite extension of the mammary growth span was accomplished by transformation of the normal cells into precancerous cell types, which grow as a benign tissue but which may occasionally undergo a second transformation into a malignant carcinoma. All precancerous tissues tested displayed unlimited growth potential, regardless of whether they occurred spontaneously or were induced by oncogenic viruses or by the administration of chemical carcinogens. Precancerous tissues of both ductal and lobuloalveolar morphology grew continuously. These results indicate that release from cell aging, as measured by the acquisition of unlimited growth potential, is associated with the precancerous state *per se*, and occurs as an early event in the transition from normal to malignant mammary cells.

0063 ELEVATED CONCENTRATIONS OF SERUM α-FETOPROTEIN IN RATS WITH CHEMICALLY INDUCED LIVER TUMORS. (Eng.) Kroes, R. (Natl. Cancer Inst., Bethesda, Md.); Sontag, J. M.; Sell, S.; Williams, G. M.; Weisburger, J. H. *Cancer Res.* 35(5):1214-1217; 1975.

Aflatoxin B_1 (AFB_1)-induced liver tumors in male Fischer 344/CS rats were used to determine whether or not an increase in concentrations of serum α-fetoprotein (AFP) would occur in congruence with the age of the animal. Liver carcinogens N-hydroxy-N-2-fluorenylacetamide (NOHFAA), N-2-fluorenylacetamide (FAA), and diethylnitrosamine were tested for their ability to induce liver tumors producing AFP. Monospecific AFP antiserum was prepared and electrophoresed to confirm tests on identity and purity. AFP was tested by bleeding the rats and separating the sera to be assayed by double diffusion in 1% agarose. Sera in experiment one were examined by double diffusion during the first ten weeks, experiment two for 30

weeks and experiment three every four weeks. Necropsies were done on all animals, and liver and grossly abnormal tissues were removed and fixed in 4% buffered formalin. AFB_1-induced liver tumors in rats revealed a definite increase in the serum concentration of AFP using the double diffusion technique. AFB_1 rats treated at six weeks of age had higher serum levels than those begun at 26 weeks of age. Radioimmunoassay showed AFP to be elevated in all tumor-bearing rats. Liver tumors were moderately well-differentiated hepatocellular carcinomas while two of the FAA-induced liver tumors and one of the NOHFAA liver tumors displaced both neoplastic hepatocytes and neoplastic bile ducts with transitional elements. Equimolar concentrations of FAA were found to be more toxic than NOHFAA and seemed to induce liver tumors that produced greater amounts of AFP. Morphological differentiation of liver carcinomas did not affect AFP production. Elevated AFP in tumor-induced rats could theoretically be used as a predictor of preneoplastic lesions. Further investigation into the pathogenicity of liver lesions in addition to the onset of elevated AFP is required to substantiate this theory.

0064 THE REGULATION OF SERINE DEHYDRATASE AND GLUCOSE-6-PHOSPHATASE IN HYPERPLASTIC NODULES OF RAT LIVER DURING DIETHYLNITROSAMINE AND *N*-2-FLUORENYLACETAMIDE FEEDING. (Eng.) Kitagawa, T. (McArdle Lab. Cancer Res., Univ. Wisconsin, Madison); Pitot, H. C. *Cancer Res.* 35(4):1075-1084; 1975.

Changes in serine dehydratase and glucose-6-phosphatase levels induced by dietary stimuli or starvation in hyperplastic nodules of male Sprague-Dawley rat liver during diethylnitrosamine or *N*-2-fluorenylacetamide feedings were studied. Rats were fed with carcinogens for either nine or 15 weeks. The study was performed during carcinogenesis through a combined method of enzyme histochemistry and radioautography. The presence or absence of induction or repression was judged by comparative study of staining patterns and their intensities between tissues from treated (induced or repressed) and nontreated (control) rats. Serine dehydratase was observed diffusely in the cytoplasm of the original hepatocytes in the periportal zone. It was induced markedly during diethylnitrosamine feeding, but only slightly during *N*-2-fluorenylacetamide feeding and was deficient and not inducible in hyperplastic nodules during their development. Later during the feeding period, an elevated serine dehydratase level, inducible with time, was found in the majority of nodules. Good correlation was observed between serine dehydratase and glucose-6-phosphatase in their elevated levels and responses to environmental stimuli. A minor group of hyperplastic nodules in which induction was not observed showed a persistent deficiency of both enzymes. Greater numbers of hyperplastic nodules with this deficiency were seen during diethylnitrosamine carcinogenesis. These results provide further information about the changing biological nature of hyperplastic nodules with respect to their metabolic adaptability and enzyme levels during hepatocarcinogenesis. The real nature of the nodules in relation to carcinoma development remains obscure.

0065 TRANSFORMING POTENTIAL OF THE ANTICANCER DRUG ADRIAMYCIN. (Eng.) Price, P. J. (Microbiol. Assoc., Bethesda, Md.); Suk, W. A.; Skeen, P. C.; Chirigos, M. A.; Huebner, R. J. *Science* 187(4182):1200-1201; 1975.

A Fischer rat embryo cell system *in vitro*, which had been shown to be highly accurate in identifying chemical carcinogens and to have application in the study of chemicals having anticancer properties, was used to study the anticancer drug adriamycin. The maximum nontoxic dose of adriamycin was found to be 0.15 mg/ml. Cells were also treated with 3-methylcholanthrene (3MC) at a concentration of 0.1 μg/ml. In two experiments, adriamycin, 3MC alone, and 3MC in combination with adriamycin, transformed the test cells (as judged by the production of progressively growing foci of cells lacking contact inhibition and orientation). When inoculated s.c. into newborn Fischer rats, 1×10^6 transformed cells produced progressively growing undifferentiated fibrosarcomas. It is suggested that the reemergence of cancer cells in humans several yr after chemotherapy may, in some cases, be due to induction by the treatment.

0066 AUTOXIDATION OF PHORBOL ESTERS UNDER NORMAL STORAGE CONDITIONS. (Eng.) Schmidt, R. (Deutsches Krebsforschungszentrum Institut fur Biochemie Im Neuenheimer Feld 280 D-69 Heidelberg, Federal Republic of Germany); Hecker, E. *Cancer Res.* 35 (5):1375-1377; 1975.

Autoxidation of diterpene phorbol esters under normal storage conditions was investigated to determine the products produced from the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and 4α-phorbol-12-13-diacetate. Solutions of TPA in acetone, ethyl acetate or methylene chloride were stored at 25 C in diffuse daylight, 4 C and -20 C respectively in the dark. Autoxidation of phorbol esters under normal storage conditions revealed appreciable amounts of autoxidation products with solutions stored at 25 C with β-epoxide *3* being the main product formed followed by autoxidation of ethyl acetate and methylene chloride at a slower rate. Solutions kept at 25 C in the dark reduced the extent of autoxidation. At 4 C only traces of autoxidation occurred. A solution of dimethyl sulfoxide (DMSO) (25 C in diffuse daylight) exhibited small amounts of aldehyde 2,$\Delta^{5,6}$-7-hydroxy compound *5* after three months with only trace amounts of ketone *6*. No degradation of TPA occurred with DMSO solutions frozen at -20 C in the dark. The only conversion product produced after storing 4α-phorbol-12,13-diacetate in the dark was 20-aldehyde. Dye-sensitized photooxygenation of TPA using a hematoporphyrin-pyridine solution and fluorescent light yields ketone *6* as the only product after refluxing of the solution. The results suggest that TPA should be stored either as a solid or in solution at -20 C in the dark. Water-saturated diethyl ether as a solvent is recommended for checking the purity of TPA.

0067 NEAR-ULTRAVIOLET LIGHT-INDUCED STRAND BREAKS IN DNA PRETREATED WITH THE CARCINOGEN, *N*-ACETOXY-2-ACETYLAMINOFLUORENE. (Eng.) Deering, R. A. (Dep. Biophys., Pennsylvania State Univ., Univ. Park); Taylor, W. D.; Burns, L. R. *Biophys. J.* 15(3):181-190; 1975.

Neutral sucrose gradients of supercoiled DNA (ϕX-174 RF I) were used to measure the *in vitro* production of strand breaks by *N*-acetoxy-2-acetylaminofluorene (AcO-AAF), AcO-AAF followed by alkali (NaOH) treatment, 303-nm ultraviolet (UV) exposure subsequent to AcO-AAF pretreatment, and alkali treatment of the UV-irradiated AAF-DNA complex. Treatment with AcO-AAF in 10% dimethyl sulfoxide did not directly yield strand breaks, but breaks in relatively low yield appeared after alkali treatment (pH 13 for 60 min) of the RF I previously treated with AcO-AAF. DNA treated with AcO-AAF was also sensitive to single-strand breakage by 303 nm near-UV light under neutral conditions, greater exposure to AcO-AAF being correlated with a higher sensitivity to 303 nm UV light. Post-irradiation alkali treatment greatly enhanced the UV light-induced rate of strand breakage. The results suggest that there are no major or minor AcO-AAF reaction products which would directly yield DNA strand breaks at neutral pH. It is also probable that only a very small fraction of the bound -AAF residues serve as alkali-sensitive sites and that the alkali-labile lesion may be a minor reaction product or one occurring at a specific site in the RF DNA. The synergistic effects of near-UV light on AcO-AAF-treated DNA and cells must be considered when evaluating molecular and cellular responses to this chemical.

0068 THE EFFECT OF MODIFICATION OF T7 DNA BY THE CARCINOGEN *N*-2-ACETYLAMINOFLUORENE: TERMINATION OF TRANSCRIPTION *IN VITRO*. (Eng.) Millette, R. L. (Univ. Colorado Med. Cent., Denver); Fink, L. M. *Biochemistry* 14(7):1426-1432; 1975.

To study the effects of *N*-2-acetylaminofluorene (AAF) modification of DNA on transcription, purified DNA from bacteriophage T7 was modified *in vitro* to varying extent with AAF and transcribed by DNA-dependent RNA polymerase from *Escherichia coli* K12. T7 was chosen as a substrate for modification because its *in vitro* transcription product was predominantly a single RNA species. The main effects of AAF modification on transcription was a marked inhibition of the rate and extent of RNA synthesis with relatively little effect on initiation except at very high AAF doses. Calibration of the percent modification with [^{14}C]AAF and analysis of the size of RNA product by double isotope labeling and polyacrylamide gel electrophoresis supported the following mechanism of transcription: most of the AAF residues bound to the coding strand of DNA cause premature termination of transcription at or near the site of modification with release of RNA polymerase. Chain termination was a linear function of drug dose. Premature termination resulted in the production of shorter RNA chains with increased amounts of bound carcinogen. The data was consistent; there was no reinitiation and/or synthesis of RNA distal to the AAF-modification site. These studies support a base displacement model of AAF binding in which the modified bases are shifted out of the double helix while the covalently bound carcinogen is inserted.

0069 INDUCTION OF ENZYMES BY GLUCAGON, GLUCOSE REPRESSION, AND ADENOSINE 3',5'-MONOPHOSPHATE CONCENTRATION DURING CARCINOGENESIS AND IN MORRIS 9618A HEPATOMA. (Eng.) Sudilovsky, O. (Inst. Pathol., Case West. Reserve Univ., Cleveland, Ohio); Gunter, R. *Cancer Res.* 35(4):1069-1074; 1975.

Glucagon induction of enzymes (serine dehydratase and tyrosine aminotransferase), cyclic AMP levels, and glucose repression of enzyme induction were studied in Morris 9618A hepatoma in male Buffalo rats and in the livers of young male Sprague-Dawley rats fed a carcinogenic diet (2-acetylaminofluorene or 3'-methyl-4-dimethylaminoazobenzene) for up to five weeks. The carcinogenic diet did not alter the basal levels of the enzymes, but the response to a single dose of glucagon was reduced. Concentrations of cyclic AMP, both basal and glucagon-induced, were not affected by the diet, nor were they different in Morris hepatoma tissue from normal liver. Morris 9618A hepatoma tissue contained only about half of the tyrosine aminotransferase of normal liver tissue, and glucagon induction of this enzyme was not repressed by glucose as was normal liver tissue. Glucose did not suppress the increased activity of serine dehydratase and tyrosine aminotransferase resulting from a single dose of glucagon in the livers of the rats fed the carcinogenic diets. This loss of glucose repression was apparent as early as 2-3 weeks. It is concluded that changes in the concentration of cyclic AMP are not the cause of alterations in enzyme induction during chemical carcinogenesis, and that the early disappearance of the glucose effect, which persists in slow-growing hepatomas, may be an expression of interference by carcinogens with the translation apparatus of the hepatic cell.

0070 AMMONIUM HYDROXIDE TREATMENT OF AFLATOXIN B_1. SOME CHEMICAL CHARACTERISTICS AND BIOLOGICAL EFFECTS. (Eng.) Vesonder, R. F. (Agric. Res. Serv., U. S. Dep. Agric., Peoria, Ill.); Beckwith, A. C.; Ciegler, A.; Dimler, R. J. *J. Agric. Food Chem.* 23(2):242-243; 1975.

Some chemical and biological properties of the reaction product(s) of 8-18 day incubations of aflatoxin B_1 with 17 N ammonium hydroxide at room temperature are reported. Thin layer chromatography and infrared and UV emission spectroscopy indicated only aflatoxin B_1 to be present after 21 hr incubation. However, when the reaction mixture was taken to dryness after 18 days of incubation, approximately 50% of the solid isolated was a mixture of aflatoxin B_1 and of the substituted *o*-coumaric acid, and the other 50% was a brown, acetone-insoluble solid which was soluble in water and methanol. This residue absorbed at 265 nm in UV and had a broad plateau from 300-362 nm; when excited at 360 nm it fluoresced at 445 nm. When tested for toxicity on fertile white

Leghorn eggs, no mortality or teratogenic effects were observed after 20 days at levels of 0.31, 0.031, and 0.005 µg per egg.

0071 AFLATOXIN B_1 HYDROXYLATION BY HEPATIC MICROSOMAL PREPARATIONS FROM THE RHESUS MONKEY. (Eng.) Krieger, R. I. (Dept. Environ. Toxicol., Univ. California, Davis); Salhab, A. S.; Dalezios, J. I.; Hsieh, D. P. H. *Food Cosmet. Toxicol.* 13(2);211-219; 1975.

The basic biochemical properties of the monkey-liver enzyme systems that catalyze the hydroxylation of aflatoxin B_1 to aflatoxins M_1 and Q_1 are reported. Cell-free liver preparations from rhesus monkeys were fortified with NADPH and incubated with labeled aflatoxin B_1. Aflatoxin Q_1 was the primary metabolite, with smaller amounts of aflatoxin M_1 and three unidentified organosoluble metabolites also formed. The enzyme systems involved were localized in the microsomal fraction and required NADPH and molecular oxygen for maximum catalytic activity. Both hydroxylase systems were inhibited by carbon monoxide and by SKF 525-A. On the basis of these biochemical properties, the aflatoxin B_1 hydroxylases of monkey liver can be classified as typical mixed-function oxidases. It is suggested that since aflatoxin B_1 is rapidly metabolized to the less toxic aflatoxin Q_1 by monkeys, they may be more susceptible to its acute toxicity and less vulnerable to its chronic or carcinogenic effects than animals which metabolize the compound more slowly. Similarities of aflatoxin metabolism in man and monkeys were discussed.

0072 EFFECT OF AFLATOXINS ON OXIDATIVE PHOSPHORYLATION BY RAT LIVER MITOCHONDRIA. (Eng.) Ramachandra Pai, M. (Vallabhbhai Patel Chest Inst., Univ. Delhi, India); Jayanthi Bai, N.; Venkitasubramanian, T. A. *Chem. Biol. Interact.* 10(2):123-131; 1975.

The *in vitro* effects of aflatoxins M_1, B_1, and G_1 on oxidation and phosphorylation by rat liver mitochondria were studied. Liver mitochondria isolated from nonfasted albino rats were incubated with the aflatoxins dissolved in DMSO, using succinate as substrate. Respiration and phosphorylation were measured polarographically at 30 C. All of the aflatoxins inhibited electron transport at concentrations of 10^{-4} M. The site of inhibition was between cytochrome b and cytochrome c or c_1. In addition, aflatoxin M_1 uncoupled oxidative phosphorylation at 10^{-6} M and reduced the ADP:O ratio, while aflatoxin B_1 (10^{-6} M) uncoupled oxidative phosphorylation, but had no effect on the ADP:O ratio. The ADP:O ratio was decreased by aflatoxin B_1 (10^{-5} M). Aflatoxin G_1 was able to uncouple oxidative phosphorylation only at relatively high concentrations (10^{-4} M). Preincubation of rat liver mitochondria with these toxins resulted in respiratory inhibition and uncoupling. It is likely that the aflatoxins exert their uncoupling effect *via* binding at specific sites responsible for oxidative phosphorylation, although they may instead effect a gross change in the structure of the mitochondrial membrane.

0073 *N*-SUBSTITUTION OF CARBON 8 IN GUANOSINE AND DEOXYGUANOSINE BY THE CARCINOGEN *N*-BENZOYLOXY-*N*-METHYL-4-AMINOAZOBENZENE *IN VITRO*. (Eng.) Lin, J. K. (Univ. Wisconsin Med. Cent., Madison); Schmall, B.; Sharpe, I. D.; Miura, I.; Miller, J. A.; Miller, E. C. *Cancer Res.* 35(3):832-843; 1975.

Chemical, radiochemical, and spectroscopic studies demonstrated that the major nucleoside-dye products formed by the reaction of *N*-benzoyloxy-*N*-methyl-4-aminoazobenzene (*N*-benzoyloxy-MAB) with guanosine and deoxyguanosine are *N*-(guanosin-8-yl)- and *N*-(deoxyguanosin-8-yl)-*N*-MAB. The presence of equimolar amounts of both MAB and guanosine or deoxyguanosine residues was shown by the $^3H:^{14}C$ ratios of the products from the reaction of prime ring-^3H-*N*-benzoyloxy-MAB with [8-^{14}C]-guanosine or [8-^{14}C]-deoxyguanosine, and by the molecular weight of the trimethylsilyl derivatives of both products. Substitution of the dye residue on its amino nitrogen was indicated by the retention in the products of the $^3H:^{14}C$ of [$CH_2{}^3H$ + $^{14}CH_2$]-*N*-benzoyloxy-MAB and by the release of MAB on treatment of the nucleoside-dye derivatives with strong alkali in air. Substitution of the guanine residues in position 7 or 8 was demonstrated by loss of ^3H from [8-^3H]-guanosine or [8-^3H]-deoxyguanosine in the formation of the nucleoside-dye derivatives. The stability of the products to mild alkali (as contrasted to the lability of 7-alkylguanosines) provided strong evidence that the substitution was in position 8 of the guanine residue. Direct evidence of 8-substitution came from the acid hydrolysis of guanosinyl- and deoxyguanosinyl-MAB to *N*-(guan-8-yl)-MAB in up to 50% yield. Comparisons of the proton or ^{13}C nuclear magnetic resonance spectra or both of *N*-(guan-8-yl)-MAB, MAB, *N*-(guanosin-8-yl)-2-acetylaminofluorene, 2-acetylaminofluorene, guanosine, and 7-methylguanosine with the spectra of the guanosine-MAB product further confirmed that the substitution had occurred at position 8 of the guanosine residue. The new compound *N*-(guan-8-yl)-MAB was synthesized. Attempts to devise an unambiguous synthesis of *N*-(guanosin-8-yl)-MAB were not successful.

0074 IMMUNOFLUORESCENT STUDY ON α-FETOPROTEIN-PRODUCING CELLS IN THE EARLY STAGE OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE CARCINOGENESIS. (Eng.) Dempo, K. (Sapporo Med. Coll., Japan); Chisaka, N.; Yoshida, Y.; Kaneko, A.; Onoe, T. *Cancer Res.* 35(5):1282-1287; 1975.

An immunofluorescence study on α-fetoprotein (AFP)-producing cells in the early stage of 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) carcinogenesis in male Wistar rats was investigated. Light microscopy was used to observe hepatic tissues fixed in Carnoy's solution with paraffin sections stained with hematoxylin and eosin, periodic acid-Schiff or toluidine blue. AFP was tested using double diffusion in agar gel with specific antisera. Tissues were histologically prepared and incubated with fluorescein isothiocyanate-labeled anti-rabbit IgG antiserum followed by absorption with acetone-extracted rat liver powder for the immunofluorescene antibody assay.

Immunofluorescence of AFP produced in early stages of 3'-Me-DAB revealed a degenerative alteration in hepatocytes after 2-3 weeks of 3'-Me-DAB ingestion. Small basophilic cells were frequently observed among or adjacent to oval cells after 4-7 weeks; they were identical with transitional cells. It was at this period that AFP was detected in the sera of rats. After eight weeks, hepatocytes increased in size and occupied hepatic lobules. In the majority of cases AFP was not detectable by the micro-Ouchterlony method. There were no cells specifically fluorescent to AFP observed in the liver tissues of normal adult rats. After four weeks, when the cells with more abundant cytoplasm than that of the early oval cells were frequently observed, a large number of cells were seen by bright fluorescence. Fluorescent cells were not observed in megalocytic hepatocytes, bile ducts or inflammatory tissue. In the liver a small number of hepatocytes were fluorescent and easily detected. The results indicate the presence of transitional cells in the precancerous stage of azo dye carcinogenesis.

0075 STRUCTURES OF HEPATIC NUCLEIC ACID-BOUND DYES IN RATS GIVEN THE CARCINOGEN *N*-METHYL-4-AMINOAZOBENZENE. (Eng.) Lin, J. K. (Univ. Wisconsin Med. Cent., Madison); Miller, J. A.; Miller, E. C. *Cancer Res.* 35(3):844-850; 1975.

Male albino rats were given a single i.p. dose (12 mg/100 g) of the hepatocarcinogenic dye *N*-methyl-4-aminoazobenzene, labeled in the prime ring with ^3H (5.7 mCi/mg). The hepatic ribosomal RNA and DNA from these rats were hydrolyzed to yield nucleoside-dye products. The major nucleoside-dye derivatives were cochromatographed with synthetic *N*-(guanosin-8-yl)- and *N*-(deoxyguanosin-8-yl)-*N*-methyl-4-aminoazobenzene, resp., on cellulose and silica gel thin layers in several solvent systems. Additional evidence for the characterization of the hepatic RNA-nucleoside derivative was obtained through its degradation by alkali in air to *N*-methyl-4-aminoazobenzene and 4-aminoazobenzene, as previously described for *N*-(guanosin-8-yl)-*N*-methyl-4-aminoazobenzene. The same nucleoside derivatives were also obtained from ribosomal RNA and DNA reacted *in vitro* with the carcinogenic electrophilic derivative *N*-benzoyloxy-*N*-methyl-4-aminoazobenzene. The data suggest that nucleic acid-bound dyes in the livers of rats given *N*-methyl-4-aminoazobenzene may arise *via* *N*-hydroxylation of *N*-methyl-4-aminoazobenzene, esterifications of the *N*-hydroxy derivative, and reaction of the resultant electrophilic esters with DNA and RNA.

0076 THE ABSORPTION OF ARSENIC AND ITS RELATION TO CARCINOMA. (Eng.) Bettley, F. R. (Middlesex Hosp., London, England); O'Shea, J. A. *Br. J. Dermatol.* 92(5):563-568; 1975.

Individual differences in the absorption, blood levels and excretion of a test dose of arsenic were studied in human subjects. On the first day four patients with arsenical carcinomas and three normal subjects were given a standard dose equivalent to 8.25 mg of arsenic in three divided doses at eight-hr intervals. Blood arsenic levels and total fecal and urinary excretion were measured daily for 7-10 days. Fecal excretion results were inconclusive; but blood levels and urinary excretion were lower in the carcinoma subjects. The difference between the normal and the test group was significant for the 8-10 day total excretion of arsenic *via* urine. Average normal excretion was 4.4 mg of arsenic. In the carcinoma patients the average was 2.9 mg. These results suggest increased storage in subjects with carcinoma. Hence, abnormally high retention of arsenic may be an individual metabolic trait and may be an important factor in carcinogenesis.

0077 TISSUE-SPECIFIC DNA-PROTEIN COMPLEXES DURING AZO DYE HEPATOCARCINOGENESIS. (Eng.) Chiu, J. F. (M. D. Anderson Hosp. Tumor Inst., Houston); Hunt, M.; Hnilica, L. S. *Cancer Res.* 35(4):913-919; 1975.

The carcinogen *N*, *N*-dimethyl-*p*-(*m*-tolylazo)aniline was fed to Fischer rats in order to study immunological specificity of nonhistone protein (NP)-DNA complexes and their changes during azo dye hepatocarcinogenesis. Immunospecificity of the protein: DNA complexes was determined by a microcomplement fixation method. Administration of *N*,*N*-dimethyl-*p*-(*m*-tolylazo)aniline changed the immunological specificity of the chromosomal nonhistone protein complexes with DNA of the same species from the type character of normal liver tissue to a new type, common to several experimental tumors, including hepatomas. The change in immunospecificity occurred early in the feeding schedule (about two weeks) and paralleled, to some extent, the increase of α-fetoprotein (determined by radioimmunoassay) in the sera of experimental animals. *In vitro* reconstitution of chromatin protein fractions showed that the immunological tissue specificity of chromatin protein:DNA complexes depends on the presence of a protein faction that binds only to homologous DNA. Immunological tissue specificity was transferred from one chromatin preparation to another by reconstituting this protein fraction to the DNA and to the remaining chromatin components.

0078 ATTEMPTS TO INDUCE TUMOURS WITH NUCLEIC ACID PREPARATIONS FROM *AGROBACTERIUM TUMEFACIENS*. (Eng.) Phillips, R. (Dep. Bot., Microbiol., Univ. College, London, England); Butcher, D. N. *J. Gen. Microbiol.* 86(2):311-318; 1975.

The tumorigenic action of nucleic acid preparations from *Agrobacterium tumefaciens* was tested using carrot root explants, sunflower and tobacco stem segments, callus cultures of sunflower, and tobacco, carrot and sunflower stems. The plant tissues were treated with phenol-purified bacterial DNA and with less highly purified preparations, including a mixture of RNA and DNA and a crude bacterial lysate containing nucleic acids, proteins, and polysaccharides; control tissues were treated with sodium citrate buffer. The capacity of the carrot root explants to degrade exogenously-applied DNA was also studied. None of the nucleic acid preparations

tested produced tumorous tissue in the carrot root explants, the principal test system; attempts to induce tumors in the callus tissues of sunflower, tobacco, and carrot were equally unsuccessful, as were attempts to produce tumors in tobacco internode segments, sunflower hypocotyl segments, cylindrical carrot phloem explants, and tobacco internode segments. These results were contrary to those obtained by earlier investigators, possibly because the latter failed to ensure that the DNA samples used were free of viable bacterial cells.

0079 CARBAMYLHYDRAZINE HYDROCHLORIDE AS A LUNG AND BLOOD VESSEL TUMOUR INDUCER IN SWISS MICE. (Eng.) Toth, B. (Univ. Nebraska Med. Cent., Omaha); Shimizu, H.; Erickson, J. *Eur. J. Cancer* 11(1):17-22; 1975.

The tumorigenic effects of carbamylhydrazine hydrochloride (CH), administered p.o., were studied in randombred Swiss mice. CH was added as a 0.0625% solution to the drinking water of male and female mice which were six weeks old at the beginning of the experiment; drug treatment was continued throughout the lives of the mice, the average daily intake of CH being 3.3 mg in the females and 4.8 mg in the males. Drug treatment somewhat shortened the lives of the experimental mice and increased the incidence of lung and blood vessel tumors. Lung tumors developed in 50% of the treated females, a total of 31 adenomas and six adenocarcinomas being diagnosed. The average age at death was 89 weeks, the first tumor being found at 50 weeks. In the treated males, 30% developed a total of 13 adenomas and eight adenocarcinomas. The average age at death was 75 weeks, the first tumor being found at 20 weeks. Eighteen percent of the females and 6% of the males developed blood vessel tumors, angiosarcomas of the liver, ovaries, or lung; angiomas of the ovaries or lymph nodes were frequently observed. The incidence of malignant lymphomas was significantly reduced in the treated mice compared with the controls, probably due to the decreased life span of the former. These results demonstrate the tumorigenicity of CH in mice and support previous studies in suggesting some sort of organ- and tissue-specific neoplastic action of the substituted hydrazines as a class.

0080 CYCLOHEXIMIDE INDUCTION OF XENOTROPIC TYPE C VIRUS FROM SYNCHRONIZED MOUSE CELLS: METABOLIC REQUIREMENTS FOR VIRUS ACTIVATION. (Eng.) Greenberger, J. S. (Natl. Cancer Inst., Bethesda, Md.); Aaronson, S. A. *J. Virol.* 15(1):64-70; 1975.

The metabolic requirements for activation of endogenous type C virus from BALB/c mouse cells by cycloheximide, a protein synthesis inhibitor, and by iododeoxyuridine, a halogenated pyrimidine, were studied. Treatment of actively replicating K-BALB cells with 10 μg cycloheximide/ml for 18 hr resulted in 2.1% registering as virus induced. In contrast, less than 0.002% became virus positive when the same cells were treated at confluence. Results with iododeoxyuridine were similar. However, cells are maximally sensitive to the actions of each drug at different times within the cell cycle. Virus induction in response to each agent is differentially inhibited under conditions of simultaneous cell exposure to inhibitors of DNA or RNA synthesis. While virus induction by cycloheximide was relatively insensitive to inhibition of DNA synthesis, its activation by iododeoxyuridine was very sensitive. These results provide support for the concept that inhibitors of protein synthesis and halogenated pyrimidines act by different mechanisms to induce type C virus release. This may mean that the two agents act at different steps in the induction pathway or that their effects on the same step are achieved by different metabolic routes.

0081 THE RELATIONSHIP BETWEEN METABOLISM, DNA BINDING, AND CARCINOGENICITY OF 15, 16-DIHYDRO-11-METHYLCYCLOPENTA[a]PHENANTHREN-17-ONE IN THE PRESENCE OF A MICROSOMAL ENZYME INHIBITOR. (Eng.) Combs, M. M. (Imp. Cancer Res. Fund Lab., London, England); Bhatt, T. S.; Vose, C. W. *Cancer Res.* 35(2):305-309; 1975.

The 7,8-benzoflavone inhibition of skin cancer induction by 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one and the influence of the flavone on the *in vitro* metabolism and binding of this carcinogen to DNA in the presence of microsomes prepared from the livers of untreated rats were investigated. The mean latent period for skin tumor production by the carcinogen in the mouse was 30 wk for a topical dose of 60 μg/wk and about 45 wk for 6 μg/wk, while at 0.6 μg/wk, no tumors were observed during 100 wk. Simultaneous administration of a closely related noncarcinogen at 54 μg/wk together with the carcinogen at 60 μg/wk had no effect on the mean latent period. Simultaneous administration of a three-fold quantity of 7,8-benzoflavone with the carcinogen at the highest dose increased the mean latent period to 38 wk, while at the intermediate dose it completely suppressed tumor formation. Neither the carcinogen nor the noncarcinogen bound covalently to calf thymus DNA *in vitro* without prior metabolic activation. After incubation with rat liver microsomes and nicotinamide adenine dinucleotide phosphate in the presence of air, both compounds bound covalently to added DNA *in vitro*, the noncarcinogen about four times more extensively than the carcinogen, roughly in proportion to the overall extent to which these ketones were metabolized. In contrast, overall metabolism of the carcinogen was increased by the addition of a three-fold quantity of the inhibitor to the incubation mixture, but binding to added DNA was almost completely prevented. These results suggest that the reactive form of the carcinogen is generated by metabolism, because no binding to added DNA occurred *in vitro* in the absence of active microsomes. If the inhibitor functions by diminishing the amount of proximate carcinogen formed in this way and available for covalent binding, it does not do so by generally inhibiting metabolism, for enhancement of the overall metabolism was observed.

0082 THE STATE OF MESSENGER RIBONUCLEIC ACID AND RIBOSOMES IN THE CYTOPLASM OF ETHIONINE-TREATED RAT LIVER. (Eng.) Endo, Y. (Sch. Med., Tokushima Univ., Japan); Tominaga, H.; Natori, Y. *Biochim. Biophys. Acta* 383(3):305-315; 1975.

Ethionine, which causes the breakdown of hepatic polysomes, was administered to female albino Wistar rats to examine the state of messenger RNA (mRNA) and monomeric ribosomes after polysome dissociation. The mRNA was selectively labeled with [^{14}C]orotate one hr after a low dose of actinomycin D (i.p., 0.55 mg/kg body weight). One hr following [^{14}C] administration, 8 ml of DL-ethionine (220 mg) was injected i.p. Sucrose density gradient centrifugation of Triton *x*-100-treated cytoplasm revealed an accumulation of heterodisperse radioactive material with very large S values which could be converted to smaller S values by deoxycholate treatment. The nature of the radioactive materials was investigated by the Millipore filter binding technique. The radioactive material was extremely sensitive to mild ribonuclease treatment. It was identified as mRNA-containing ribonucleoprotein particles because it was banded at around 1.43 g/cm^3 in CsCl gradient centrifugation and contained RNA with a distribution of S values characteristic of polysomal mRNA. Monomeric ribosomes dissociated into subunits in the presence of 0.5 M KCl; it thus appears that the ribosomal monomers in the ethionine-treated livers contain no peptidyl-transfer RNA. In rats recovered from ethionine treatment by administration of adenine and methionine, the heterodisperse ribonucleoprotein particles and monomeric ribosomes appeared to be utilized for the reformation of polyribosomes. It is thus demonstrated that mRNA exists as ribonucleoprotein in the cytoplasm of ethionine-treated liver cells.

0083 BIOCHEMICAL AND STRUCTURAL CHANGES OF RAT LIVER LYSOSOMES BY ETHIONINE. (Eng.) Zuretti, M. F. (Instituto di Patologia Generale, Universita di Torino, Corso Raffaello 30, I-10125 Torino, Italy); Baccino, F. M. *Exp. Mol. Pathol.* 22(2):271-283; 1975.

The effect of ethionine administration i.p. (1 g/kg) on the biochemical and structural characteristics of liver lysosomes of male and female Wistar rats was investigated. After ethionine administration, the free activity of the lysosomal marker acid phosphatase in liver homogenates was unchanged after three hr, consistently increased at six hr, and returned to normal by 13 hr. The free activity of β-N-acetylglucosaminidase was similarly increased at six hr. The proportion of acid phosphatase activity recovered in the soluble phase of the homogenate was in most cases significantly increased six hr after ethionine. The levels of soluble activity were distinctly lower than those for free activity. The ratio of free to total activity for acid phosphatase and β-N-acetylglucosaminidase was not modified by varying the substrate concentration in the medium, thus ruling out an acquired permeability of the lysosomal membrane to the substrates as a likely mechanism for the increased free activity. The fragility of lysosomes six hr after ethionine was investigated by exposing the whole homogenate to mechanical or hypoosmotic shock or preincubation at pH 5, 37 C. In all cases both hydrolases were activated to a higher degree than control preparations. An enhanced osmotic fragility of lysosomes in liver homogenates was also observed at three and 13 hr after ethionine. These findings suggest the presence of an increased proportion of particularly fragile particles in the liver of ethionine-treated animals. The increased proportion of acid phosphatase activity in whole homogenates may be accounted for by three possibilities: 1) by phenomena of secondary adsorption onto or entrapment by sedimentable material, after prior release from particles damaged during the homogenization; 2) by an injury to the lysosomal membrane that would allow free entry of the substrate molecules, but no solubilization of the enzymes; or 3) by the appearance of a particle unusually permeable to β-glycerophosphate and *p*-nitrophenyl glucosaminide. It is speculated that the derangement of some energy-requiring process, resulting from the previously documented marked ATP depletion caused by ethionine might play a role in causing the lysosomal changes.

0084 A RESTROSPECTIVE CASE-CONTROL STUDY OF DISEASES ASSOCIATED WITH ORAL CONTRACEPTIVE USE. (Eng.) Greenblatt, D. J. (Boston Collab. Drug Surveill. Program, Mass.). *Am. Heart J.* 89(5): 677-678; 1975.

A case-control study of diseases associated with the use of oral contraceptives was undertaken in 5,472 women diagnosed as having idiopathic venous thromboembolism and gallbladder diseases. Eight hundred and forty-two premenopausal women free of any chronic diseases and hospitalized only for acute illnesses (trauma, respiratory infections, gastroenteritis and appendicitis) or elective surgery were selected as control patients. Venous thromboembolism in association with oral contraceptive use was found to be highly significant, with duration of use having no influence on the risk of contracting thromboembolism. Coagulability enhancement produced by the use of oral contraceptive is thought to be the reason for the association of venous thromboembolism with oral contraceptive usage. Gallbladder disease was found to develop during the first year of oral contraceptive use. Benign breast tumors were found to be unassociated with the use of oral contraceptives. This study suggests oral contraceptives to be a therapeutic agent against the development of benign breast tumors. Only association between certain disease states and oral contraceptives may be deduced from this study.

0085 PRENATAL EXPOSURE TO STILBESTROL. A PROSPECTIVE COMPARISON OF EXPOSED FEMALE OFFSPRING WITH UNEXPOSED CONTROLS. (Eng.) Herbst, A. L. (Massachusetts Gen. Hosp., Boston); Poskanzer, D. C.; Robboy, S. J.; Friedlander, L.; Scully, R. E. *N. Engl. J. Med.* 292(7):334-339; 1975.

The effects of prenatal exposure to diethylstilbestrol were studied through a prospective investigation of 110 exposed and 82 unexposed females. The results of epidemiologic, clinical, and pathological investigations were compared. The drug had been administered to the subjects' mothers according to a standard schedule, with the dosage depending on the week in pregnancy in which the therapy was started. During or before the sixth week, 2.5 mg daily was given; the dosage increased to a maximum of 150 mg and was discontinued at the end of the 35th week. Of the

exposed women who agreed to be examined, 66% were 24-25 yr old, the remainder being 18-23. Among the exposed subjects, there were striking benign alterations of the genital tract, which included transverse ridges (22%), abnormal vaginal mucosa (56%), and biopsy-proved adenosis (35%). Among the unexposed women, there were no ridges. Abnormal cervical epithelium occurred in almost all exposed subjects, but in only half of the unexposed subjects. The incidence of vaginal adenosis was highest when diethylstilbestrol was begun in early pregnancy. It was not detected when treatment was initiated in the 18th week or later. Oral contraceptive use and prior pregnancy were associated with less adenosis and erosion, resp. No cases of cancer were observed. These findings suggest that the pathogenesis of cervical erosion and vaginal adenosis may not be identical, and that the two processes may differ in biologic behavior on long-term investigation. These results are in agreement with others which show that non-neoplastic cervical and vaginal abnormalities are common in the population prenatally exposed to diethylstilbestrol.

0086 ON THE MECHANISM OF HORMONE ACTION IN 7,12 DIMETHYLBENZ(A)ANTHRACENE-INDUCED MAMMARY TUMOR. I. PROLACTIN AND PROGESTERONE EFFECTS ON ESTROGEN RECEPTOR *IN VITRO*. (Eng.) Sasaki, G. H. (Univ. Oregon Med. Sch., Portland); Leung, B. S. *Cancer* 35(3):645-651; 1975.

The presence of estrogen receptors (ER) and the influence of various hormones on the binding capacity of these receptors was studied in 7,12 dimethylbenz(a)anthracene (DMBA)-induced mammary tumors from female Sprague-Dawley rats. Dextran-charcoal assay, Sephadex chromatography, sucrose gradient sedimentation, and organ culture techniques demonstrated the cytoplasmic binding of ^{3}H-estradiol in cytosol preparations from excised tumors. Binding sites were present at 10^{-13} to 10^{-15} moles/mg protein, and the dissociation constant was in the range of 10^{-9} to 10^{-10} M. Uterine tissue had the highest concentration of ER and nontarget tissue (diaphragm) had the lowest. The presence of prolactin (5 μg/ml) and insulin (5 μg/ml) in the incubation medium significantly increased the estrogen binding capacity. This stimulation was tissue-specific (present in uterine and tumor, absent in diaphragm), and was inhibited by progesterone (1 μg/ml). Insulin and prolactin acted synergistically. It is suggested that the interaction of prolactin, estrogen, and progesterone may be a common phenomenon for all estrogen-responsive tissues.

0087 PROPERTIES, INDUCIBILITY, AND AN IMPROVED METHOD OF ANALYSIS OF ARYL HYDROCARBON HYDROXYLASE IN CULTURED HUMAN LYMPHOCYTES. (Eng.) Gurtoo, H. L. (Grace Cancer Drug Cent., Buffalo, N. Y.); Bejba, N.; Minowada, J. *Cancer Res.* 35(5):1235-1243; 1975.

Development of an improved method of analysis for properties and inducibility of aryl hydrocarbon hydroxylase (AHH) in cultured human lymphocytes was investigated. Lymphocytes were obtained from healthy volunteers followed by centrifugation which yielded cell pellets containing 95% lymphocytes. Lymphocyte yields were calculated from differential cell counts and total WBC counts. From 20 ml of blood, lymphocytes were isolated to give four final cultures (two induced and two controls). AHH activity was measured by a modified fluorometric procedure (pH 8.5 and use of higher concentrations of NADPH and NADH). Properties, inducibility, and an improved method of analysis of AHH in cultured human lymphocytes revealed no demonstrable AHH activity unless pretreated with a mitogen for conversion to lymphoblasts. AHH activity exhibited an absolute requirement for NADPH. AHH activity was inhibited after treatment with $MnCl_2$. Freezing cell pellets at -20 C resulted in a significant loss of activity, while at -70 C inhibition of this loss was reached. An increase in activity was also observed when cells were maintained at pH 8.5. 3-methylcholanthrene and dibenz(a,h)anthracene (DBA) induced the enzyme activity, with DBA being a more potent inducer. The results suggest that AHH activity is acquired only after transformation of cells to lymphoblasts after treatment with a mitogen. It is postulated that the described AHH assay offers advantages for studying the genetics and mechanism of induction of microsomal enzymes and the capacity for metabolism of drugs, steroids and carcinogenic compounds in humans.

0088 BENZPYRENE HYDROXYLASE ACTIVITY IN HEPATIC MICROSOMAL AND SOLUBILIZED SYSTEMS CONTAINING RABBIT OR RAT CYTOCHROME P-448 OR P-450. (Eng.) Philpot, R. M. (Natl. Inst. Environ. Health Sci., Res. Triangle Park, N.C.); Bend, J. R. *Life Sci.* 16(6):985-998; 1975.

Liver microsomes prepared from adult male rabbits (Dutch belt strain) and rats (CD strain) injected i.p. with 25 mg/kg of 3-methylcholanthrene (3-MC) 72 hours prior to sacrifice were studied to determine if rabbit and rat cytochrome P-448 is responsible for the observed difference in benzpyrene hydroxylase (BPOH) activity between the two species in response to 3-MC. Solubilized P-450 and P-448 fractions were prepared from microsomes by sonication, sodium cholate digestion and ammonium sulfate fractionation between 35 and 50% saturation. Radioactivity in microsomes and solubilized preparations from ^{14}C-3-MC-treated rats and rabbits was determined by liquid scintillation. The concentration of cytochrome P-448 was 1.43 nM/mg protein in treated rats *versus* 1.04 nM/mg in controls, and 3.11 nM/mg in treated rabbits *versus* 1.52 nM/mg protein in controls. BPOH activity, measured by production of 8-hydrobenzpyrene (8-OHBP), was increased in 3-MC-treated rats (0.63 nM 8-OHBP/min/nM of cytochrome *versus* 0.31 nM in controls) and decreased in treated rabbits (0.14 nM 8-OHBP/min/nM of cytochrome *versus* 0.32 nM in controls). It is suggested that there is a direct relationship between the formation of cytochrome P-448 and BPOH activity in the rat, but no effect of 3-MC on BPOH activity in the rabbit.

0089 BENZO(*a*)PYRENE EFFECTS ON MOUSE EPITHELIAL CELLS IN CULTURE. (Eng.) Bartholomew, J. C. (Lawrence Berkeley Lab., Univ.

California, Berkeley); Salmon, A. G.; Gamper, H. B; Calvin, M. *Cancer Res.* 35(3):851-856; 1975.

The effect of benzo(*a*) pyrene on the growth in culture of five mouse epithelial cell strains was examined. These epithelial cells were highly sensitive to the cytotoxic action of benzo(*a*)pyrene when measured by maximum cytotoxicity (the highest percentage of decrease in cell number relative to a control seen after three days of treatment with carcinogen) and by half-maximal cytotoxicity (µg/ml; the concentration of the carcinogen required to give 50% of the maximum cytotoxicity in the three-day assay). The activity of the benzo(*a*)pyrene-metabolizing system, aryl hydrocarbon hydroxylase, was low but highly inducible by the carcinogen. As the sensitivity of a cell strain to the cytotoxic action of benzo(*a*)pyrene decreased, the inducibility of the hydroxylase also decreased. The coefficients of linear correlation between parameters describing the cytotoxic effect and those describing enzyme activity showed a moderate positive correlation between enzyme inducibility and cytotoxicity when the values from different cells strains were compared. These correlation coefficients increased if the correlation was calculated over a more closely related group of cell strains. These results suggest that the hydroxylase is important in determining the sensitivity of epithelial cells to the cytotoxic action of benzo(*a*)pyrene, but that other factors, such as transport and further metabolism of the products, may also determine the response of a cell to the carcinogen.

0090 METABOLISM OF POLYCYCLIC HYDROCARBONS BY A HIGHLY ACTIVE ARYL HYDROCARBON HYDROXYLASE SYSTEM IN THE LIVER OF A TROUT SPECIES. (Eng.) Ahokas, J. T. (Dep. Pharmacol., Univ. Oulu, SF-90220 Oulu 22, Finland); Pelkonen, O.; Kärki, N. T. *Biochem. Biophys. Res. Commun.* 63(3):635-641; 1975.

The capacity of trout liver microsomes to convert benzo(*a*)pyrene to a typical pattern of oxidized metabolites is reported. The hydroxylating system is a typical monooxygenase system in many respects when compared with the mammalian system, needing oxygen and NADPH for full activity. Trout liver microsomes converted benzo(*a*)pyrene to dihydrodiols and hydroxymetabolites at a rate 5-10 times higher than adult male Sprague-Dawley rat liver microsomes, when measured per mg of microsomal protein. Trout liver microsomes metabolized benzo(*a*)pyrene 15-30 times as fast as rat liver microsomes when the activity was measured per unit of cytochrome 450 and NADPH-cytochrome c reductase. A previously published hypothesis postulated a specific mechanism for the initiation of cancer by polycyclic hydrocarbons which involves the metabolism of polycyclic hydrocarbons to epoxide intermediates which then combine covalently with cellular macromolecules. This study shows that the prerequisite for the activation of polycyclic hydrocarbons, a cytochrome P-450-linked monooxygenase system, is present in trout liver microsomes and is very efficient in converting benzo(*a*)pyrene to oxidized metabolites, probably via an epoxide intermediate.

0091 ARYL HYDROCARBON (BENZO[A]PYRENE) HYDROXYLASE: A MIXED-FUNCTION OXYGENASE IN MOUSE SKIN. (Eng.) Wiebel, F. J. (Natl. Cancer Inst., Bethesda, Md.); Leutz, J. C.; Gelboin, H. V. *J. Invest. Dermatol.* 64(3):184-189; 1975.

The distribution of aryl hydrocarbon hydroxylase (AHH) activity in Swiss mouse skin was studied together with the kinetics of induction and its inducibility by various compounds. AHH distribution was similar after induction of the enzyme by topical application of benzo(a)anthracene (0.3 mg) or i.p. injection (3.0 mg). Enzyme activity was highest in the superficial dermis (which contains the sebaceous glands and the upper pilary canals), intermediate in the epidermis, and lowest in the deeper dermal layers. Induction of AHH also occurred after topical application of naphthacene, 7-12-dimethylbenz(a)anthracene, 3-methylcholanthrene, 7,8-benzoflavone, and 5,6-benzoflavone. The duration and magnitude of induction, but not the initial kinetics, were dependent on the inducer dose. In the absence of exogenous reduced nicotinamide adenine dinucleotide phosphate (NADPH), hydroxylase activity was reduced by 70% in skin homogenates and almost completely abolished in lung and liver homogenates. Carbon monoxide inhibited enzyme activity in these tissues by 60%. The requirement for NADPH and the inhibition by carbon monoxide indicate that cutaneous AHH is one of the mixed-function oxygenases. Some polycyclic hydrocarbons are known to require activation by microsomal oxygenases to become cytotoxic and carcinogenic. In view of this requirement for metabolic activation, the high activity of AHH in the superficial dermis may explain two previously observed phenomena: the selective destruction of sebaceous glands by topical application of polycyclic hydrocarbons, and the correlation between the absence or reduced number of sebaceous glands and lower cutaneous tumorigenesis.

0092 INTERACTIONS OF HEME WITH HEPATIC MICROSOMAL MONOOXYGENASE. EFFECT ON BENZPYRENE HYDROXYLATION. (Eng.) Brown, J. E. (Worcester Found. Exp. Biol., Shrewsbury, Mass.); Kupfer, D. *Chem. Biol. Interact.* 10(1):57-70; 1975.

The effects of heme on the monooxygenase activity in intact and "solubilized" hepatic microsomes were studied in male Sprague-Dawley rats. The animals were injected i.p. once daily (80 mg/kg) for four days with phenobarbital (PB) or 3-methylcholanthrene (MC) (25 mg/kg). The animals were starved after the last injection and intact and solubilized liver microsomes were obtained. The microsomal preparations were incubated with heme (1-25 µl in 0.01 N NaOH), NADPH, and (^{14}C) benzpyrene (BP), after which the degree of BP-hydroxylation was determined. Heme at 1-10 µM increased the rate of microsomal BP hydroxylation by 20-40% over control levels, while protoporphyrin IX inhibited BP hydroxylation in these preparations. Heme caused no increase in enzymatic activity in solubilized liver microsome preparations, which suggested that an apo-cytochrome P-450 may have been present in the intact microsomes. Higher con-

centrations of heme inhibited BP hydroxylation by both intact and solubilized microsomes; in the solubilized preparations, the inhibition was of a noncompetitive, mixed type, but it was not possible to determine the type of inhibition in the intact preparations. The addition of heme to the liver microsomes produced a marked increase in the trough (419-420 nm) of the difference spectrum formed by the subsequent addition of BP. This suggests that heme increased the microsomal binding of BP, although it is possible that BP merely displaced the bound heme of the microsomes. In any event, it is clear that both heme and BP must have elicited a change at the binding site(s) of the liver microsomes.

0093 EFFECT OF DIMETHYL SULFOXIDE ON THE HEPATIC DISPOSITION OF CHEMICAL CARCINOGENS. (Eng.) Levine, W. G. (Albert Einstein Coll. Med., Yeshiva Univ., Bronx, N.Y.). *Ann. N.Y. Acad. Sci.* 243:185-193; 1975.

The effect of dimethylsulfoxide (DMSO) on the hepatic binding of chemical carcinogens was examined in female Wistar rats. The biliary excretion of metabolites of ^{3}H-3-methylcholanthrene (MC) was much more rapid when MC was administered in DMSO solution than when given in a 1% albumin suspension, although no difference in excretion rate was observed when DMSO alone was administered five min prior to giving MC in 1% albumin. Similar results were observed with 3,4-benzpyrene (BP), although the difference in excretion rate was less marked than with MC. Following i.v. injection of ^{3}H-MC, the binding of radioactivity by the hepatic microsomal fraction was twice as great when DMSO was the solvent as when MC was administered in 1% albumin. When MC was added to liver homogenates with DMSO as the vehicle, the binding to microsomes was about seven times that when the vehicle was 1% albumin. Similar results were observed with ^{3}H-BP. The sedimentation characteristics of hepatic organelles were not altered by DMSO. Addition of unlabeled MC in ^{14}C-DMSO to liver homogenates resulted in no binding of label to the microsomes. The altered binding of MC to liver fractions occurred only when the carcinogen was injected *in vivo* or added *in vitro* as a DMSO solution, and not when DMSO was injected or added to a homogenate five min before injection or addition of MC in 1% albumin. When added to particulate fractions of the livers from untreated animals, ^{3}H-MC bound tightly to all particulate fractions (600 x *g*, 10,000 x *g*, and 100,000 x *g*), but when added as a DMSO solution, approximately 50% of the radioactivity could be washed out of the 600 x *g* and 10,000 x *g* fractions, while the affinity for the microsomal fraction appeared to be similar to that of ^{3}H-MC in albumin. With ^{3}H-BP, tight binding to all three fractions was observed when the carcinogen was added in albumin, but in DMSO solution it had less affinity for the 600 x *g* and the 10,000 x *g* fractions than for the microsomal fraction. Thin layer chromatography demonstrated complexes formed between DMSO and each of the carcinogens, and it is concluded that the complexes have different binding affinities for the hepatic organelles than do the free carcinogens. Caution is urged in interpreting results in experimental systems in which DMSO is used as a solvent for otherwise insoluble compounds.

0094 COMPARATIVE EFFECT OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON AZO DYE METABOLISM IN RAT LIVER. II. *IN VIVO* BINDING OF METABOLITES TO CELLULAR MACROMOLECULES. (Eng.) Decloitre, F. (Institut de Recherches Scientifiques sur le Cancer, Boite Postale No. 8, 94800 Villejuif, France); Martin, M.; Chauveau, J. *Chem. Biol. Interact.* 10(5):301-307; 1975.

In vivo binding of 4-dimethylaminoazobenzene (DAB) metabolites to cellular macromolecules pretreated with phenobarbital (PB) and 3-methylcholanthrene (3-MC) on azo dye metabolism in liver of Sprague-Dawley rats was investigated. A group of 12 rats was divided into PB and control subgroups. PB in 0.6% saline was injected i.p., with control rats receiving only saline. A second group of 12 rats was divided into three subgroups with the first group designated the control group, the second subgroup receiving 3-MC dissolved in olive oil and the third subgroup of rats receiving a diet of 3-MC after the initial pretreatment. All subgroups were fed [^{14}C] DAB diet to evaluate the average intake of DAB for each subgroup. Chromatin was extracted from nuclei followed by centrifugation in CsCl. DNA-containing fractions were pooled and precipitated with an equal volume of trichloroacetic acid (TCA). To determine the radioactivity bound to proteins, total protein from the 3-MC series was solubilized in NaOH followed by utilization of the total protein with Instagel expressed in nmoles of [^{14}C] DAB metabolites bound per g of dry protein. For the PB series, total protein was solubilized in NaOH followed by determination of radioactivity of the alkaline solution with Instagel. Radioactivity bound to DNA was determined by sedimentation of nuclear DNA in TCA washed with ethanol followed by hydrolyzation with HCl. *In vivo* binding of DAB metabolites to cellular macromolecules pretreated with PB and 3-MC revealed radioactive metabolites bound to DNA to be weak in rats on a [^{14}C] DAB diet. Pretreatment with PB exhibited a decrease of metabolites bound to DNA proteins. The binding of DAB metabolites to cellular macromolecules was found to be uncorrelated to the DAB intake. DAB metabolite binding to nuclear protein increased significantly after injection of 3-MC followed by no change in the binding of DAB metabolites to total protein. The results indicate that *in vivo* DAB metabolite binding on DNA and protein is dependent on the route of administration 3-MC. It is postulated that DAB metabolites binding to cellular constituents *in vivo* results from a balance between detoxication and activation reactions. These factors suggest that several injections of PB might reduce and a single injection of 3-MC might enhance the incidence of hepatomas.

0095 THE INFLUENCE OF THYROID STIMULATION ON THE INCIDENCE OF 3-METHYLCHOLANTHRENE-INDUCED TUMORS. (Eng.) Baker, D. G. (Mt. Zion Hosp. Med. Cent., San Francisco, Calif.); Yaffe, A. H. *Cancer Res.* 35(3):528-530; 1975.

The induction of s.c. tumors at the injection site of a single dose of 3-methylcholanthrene (2 mg) was studied in euthyroid, thyroidectomized, and hyperthyroid female Sprague-Dawley rats. Ninety-two percent of euthyroid animals, 71% of thyroidectomized, and 36% of hyperthyroid rats developed tumors at the injection site, and mean survival times for the three groups were 352, 404, and 254 days, resp. The thyroidectomized and hyperthyroid rats had a slight reduction in tumor induction time compared to euthyroid rats (about 209 days compared to 281 days). Thyroid feeding increased the metabolic rate while thyroidectomy resulted in little or no reduction. Food intake was similar in the thyroidectomized and euthyroid groups, but the hyperthyroid rats had a significant increase in both food and water intake. The results indicate an inverse correlation between metabolic rate and tumor incidence but fail to show a significant effect of food intake.

0096 ALTERED GANGLIOSIDE BIOSYNTHESIS IN MOUSE CELL CULTURES FOLLOWING TRANSFORMATION WITH CHEMICAL CARCINOGENS AND X-IRRADIATION. (Eng.) Coleman, P. L. (Natl. Inst. Neurol. Dis. Stroke, Bethesda, Md.); Fishman, P.H.; Brady, R. O.; Todaro, G. J. *J. Biol. Chem.* 250(1):55-60; 1975.

Altered ganglioside patterns and enzyme activities in BALB/c 3T3 mouse embryo cells transformed by methylcholanthrene and benzopyrene and by x-irradiation are described, and the properties of the residual UDP-Gal:*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide galactosyltransferase activity in the transformed cells are compared with those of the normal cell enzyme. The transformed cells had reduced amounts of the mono- and disialogangliosides galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide and *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide, and increased amounts of *N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide. The activity of the enzyme UDP-Gal: *N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide galactosyltransferase was reduced to 2.7-14.3% of normal in the transformed clones. Other ganglioside glycosyltransferase activities were unaffected. This enzymatic change is claimed to be consistent with the observed alteration in ganglioside pattern in the transformed cells. The residual galactosyltransferase activity in the transformed cells was kinetically similar to the normal enzyme, suggesting that transformation alters ganglioside biosynthesis by blocking enzyme synthesis at the translational or transcriptional levels.

0097 FREE AND MEMBRANE-BOUND RIBOSOMES IN NORMAL AND METHYLCHOLANTHRENE-TREATED MOUSE EPIDERMIS. (Eng.) Mueller, S. N. (Dep. Biol., Syracuse Univ., New York); Argyris, T. S. *Lab. Invest.* 32(2):209-216; 1975.

The proportions of free and membrane-bound ribosomes in normal and 3-methylcholanthrene (3-MC)-induced hyperplastic mouse epidermis were compared. 3-MC (2 µM) dissolved in benzene was applied to the unshaved backs of female CD-1 mice. The epidermis from these animals and from untreated controls was trypsinized and homogenized, and the free and bound ribosomes were isolated from the postnuclear supernatant. The RNA and DNA extracted from the whole homogenates and ribosomal pellets were also analyzed and biopsy specimens from normal and 3-MC-treated whole and trypsinized skin were examined histologically. The value for the total ribosomes from the normal epidermis in the resting phase of the hair growth cycle was 0.32 mg of ribosomal RNA/g of epidermis, or 0.07 mg of ribosomal RNA/mg of homogenate DNA. About 96% of the ribosomes were free and 4% were membrane-bound. Five days after 3-MC treatment, there was a marked epidermal hyperplasia and cellular hypertrophy. The total ribosomes increased 2-3-fold, to 0.80 mg of ribosomal RNA/g of epidermis, or 0.21 mg of ribosomal RNA/mg of homogenate DNA. This increase was accompanied by proportionate increases in both free and membrane-bound ribosomes, although the increase in membrane-bound ribosomes was not statistically significant. The relative increase in ribosomes may have been related to the initial level of ribosomes within the cell.

0098 EFFECT OF A SINGLE TREATMENT WITH THE ALKYLATING CARCINOGENS DIMETHYLNITROSAMINE, DIETHYLNITROSAMINE AND METHYL METHANESULPHONATE, ON LIVER REGENERATING AFTER PARTIAL HEPATECTOMY. II. ALKYLATION OF DNA AND INHIBITION OF DNA REPLICATION. (Eng.) Craddock, V. M. (Med. Res. Counc. Lab., Carshalton, England). *Chem. Biol. Interact.* 10(5):323-332; 1975.

Experiments were carried out to determine whether replication of alkylated DNA could be involved in the initiation of hepatocellular carcinoma which is known to result from a single administration of dimethylnitrosamine (DMN) after partial hepatectomy. DNA from liver regenerating after partial hepatectomy in porton strain rats treated with DMN was isolated and analyzed. Experiments on DNA synthesis involved i.p. injection of 100 µCi [^{3}H] thymidine after partial hepatectomy followed by homogenization of the liver in perchloric acid centrifuged at 2C. Total acid-soluble tritium present in the liver was then determined. The incorporation of [^{3}H] thymidine into liver DNA was determined in intact animals. Alkylation of DNA and inhibition of DNA replication after a single treatment with the carcinogens diethylnitrosamine (DEN), dimethylnitrosamine (DMN), and methyl methanesulphonate (MMS) on liver regenerating after partial hepatectomy revealed no significant changes in the replication of DNA after administration of DMN. The disappearance of 3-methyladenine from DNA occurred during the prereplicative stage after six hr of administration of DMN or DEN. [^{3}H] thymidine incorporation remained slightly above base line values. The administration of MMS six hr after partial hepatectomy produced a delay in DNA synthesis, with the maximum rate occurring eight hr later. The administration of the nitrosamines after DNA synthesis was underway resulted in a reduction of [^{3}H] thymidine incorporation. DNA synthesis occurred at a higher level in tumor-induced animals than in intact animals. Although the nitrosamines produced an in-

hibitory effect on DNA replication following partial hepatectomy, the inhibition did not reduce DNA synthesis to the level occurring in normal intact livers. This factor suggests that replication of damaged DNA before the abnormality is repaired in necessary for the initiation of cancer.

0099 MULTIPLE MYELOMA AND ACUTE LEUKEMIA ASSOCIATED WITH ALKYLATING AGENTS. (Eng.) Kyle, R. A. (Mayo Clin., Rochester, Minn.); Pierre, R. V.; Bayrd, E. D. *Arch. Intern. Med.* 135(1):185-192; 1975.

Case histories are presented for five patients (48-67 yr, all males) with multiple myeloma who developed rapidly fatal acute myelomonocytic leukemia after treatment with melphalan for 28-54 months. All five patients had lytic bone lesions and three had compression fractures. Serum samples from four patients all showed evidence of a monoclonal protein. In each patient, multiple myeloma responded to therapy; and progress was satisfactory until the development of acute leukemia, which was fatal within a few weeks. At postmortem examination, leukemic infiltration was seen, and there was little or no evidence of myeloma. Another group of five patients was also seen in whom myeloma had responded to melphalan but in whom a persistent pancytopenia and preleukemic changes in the bone marrow developed in the absence of overt symptoms of leukemia. Consideration of these cases and a review of the literature suggest that these circumstances represent the development of acute myelomonocytic leukemia in multiple myeloma, probably related to the melphalan.

0100 ORGAN-SPECIFIC EFFECTS OF DNA METHYLATION BY ALKYLATING AGENTS IN THE INBRED SWISS MOUSE. (Eng.) Maitra, S. C. (Fac. Med., Univ. West. Ontario, London, Canada); Frei, J. V. *Chem. Biol. Interact.* 10(4):285-293; 1975.

DNA alkylation of mouse tissue with *N*-methyl-*N*-nitrosourea (MNUA) and methyl methanesulfonate (MMS) was studied to determine why the former is a potent carcinogen in mice while the latter is ineffective as a tumor-inducing agent. Inbred Swiss female mice (aged 6-8 weeks), given single or repeated equitoxic i.p. doses of MNUA or MMS, developed thymomas and pulmonary adenomas only after MNUA administration. Mice of the same strain were then injected i.p. with ^{14}C-MMS (1.2 mM, 2.1 mCi/kg) or with ^{14}C-MNUA (0.6 mM, 1.2 mCi/kg). After two or 12 hr, organs (bone marrow, thymus, spleen, liver, kidneys, lungs, and gut) were pooled for DNA extraction. Chromatography of DNA hydrolysates revealed nearly identical overall alkylation of tumor target tissues by both agents; about 80% of the alkylation resulted in the biologically ineffective product 7-methylguanine and about 10% resulted in 3-methyladenine. The main difference in DNA alkylation was the production of O^6-methylguanine (a known pre-mutagenic product) by MNUA in amounts ten or more times larger than those found following MMS administration. This supports the possibility that somatic mutations are a part of the process of carcinogenesis.

0101 EFFECT OF A SINGLE TREATMENT WITH THE ALKYLATING CARCINOGENS DIMETHYLNITROSAMINE, DIETHYLNITROSAMINE AND METHYL METHANESULPHONATE, ON LIVER REGENERATING AFTER PARTIAL HEPATECTOMY. I. TEST FOR INDUCTION OF LIVER CARCINOMAS. (Eng.) Craddock, V. M. (Med. Res. Counc. Lab., Carshalton, England). *Chem. Biol. Interact.* 10(5):313-321; 1975.

The effect of a single treatment with the alkylating carcinogens dimethylnitrosamine (DMN), diethylnitrosamine (DEN), and methyl methanesulfonate (MMS) on regenerating liver after partial hepatectomy was studied. A single treatment of intact animals with DMN did not induce liver cell cancer. Treatment after partial hepatectomy (12.0-15.6 mg/kg) caused liver cell damage, disruption of lobular architecture and development of hyperplastic nodules. Some cases exhibited definite liver cell cancer. DMN administered 24-hr after partial hepatectomy produced a higher liver tumor incidence as opposed to administration in the early prereplicative stage. Kidney tumors were also found to be higher in animals given DMN 24-hr after hepatectomy. DEN administration caused liver cell cancer in two of nine animals. These cancers were anaplastic, while the majority of those induced after partial hepatectomy were trabecular. The administration of MMS failed to induce liver cell cancer. However, one animal treated 24 hr after partial hepatectomy developed a mesenchymal kidney tumor. DMN results in the production of a carcinogenic tumor only after partial hepatectomy. It is suggested that the replication of alkylated DNA may be a significant event in carcinogenesis.

0102 CARCINOMAS OF THE ESOPHAGUS IN RATS INGESTING DIETHYLNITROSAMINE. (Eng.) Reuber, M. D. (Natl. Cancer Inst., Bethesda, Md.). *Eur. J. Cancer* 11(2):97-99; 1975.

The induction of carcinomas of the esophagus and liver was studied in male and female Buffalo rats given 0.014% diethylnitrosamine (DEN) in the diet for 26 weeks. The rats survived an average of 28 weeks. Nine out of 14 males and five out of 14 females developed carcinomas of the esophagus; the carcinomas were focal and were located either near the gastroesophageal junction or at the level of the pharyngeal-tracheal bifurcation. Ten females had carcinomas and three had sarcomas of the liver, compared with five carcinomas and two sarcomas of the liver in the males. BD rats given asymmetrical nitrosamines regularly developed localized or diffuse carcinomas of the esophagus, but carcinomas of the liver occurred only in rats given ethyl-ethanolnitrosamine. In the case of DEN, the findings indicated that the chemical was first transformed into an active carcinogenic agent in the esophageal mucosa and later reached the liver. With diffuse localization in the esophagus in rats given asymmetrical nitrosamines, it is predicted that little of the chemicals or their metabolites reach the liver.

0103 PROTECTION BY CARBON TETRACHLORIDE AGAINST THE TOXIC EFFECTS OF DIMETHYLNITROSAMINE IN MICE. (Eng.) Pound, A. W. (Dep. Pathol., Univ. Queensland, Brisbane, Australia); Lawson, T. A. *Br. J. Exp. Pathol.* 56(1):77-82; 1975.

The effect of carbon tetrachloride (CCl_4) on dimethylnitrosamine (DMN) toxicity was investigated in male Crackenbush mice. The LD_{50} of DMN increased by a factor of about 4.2 after a single i.p. dose of CCl_4 (0.5 ml/kg), and by a factor of 1.2 after an extremely small dose (0.004 ml/kg). The increase in the LD_{50} correlated with a decrease in DMN-demethylase activity in the liver of CCl_4-treated mice, enzyme activity decreasing to 73% of normal after a dose of 0.6 ml/kg of CCl_4. These effects began within ten min of CCl_4 administration, and increased very rapidly for 12 hr to a level which was maintained for up to 60 hr, after which the LD_{50} of DMN and the level of DMN-demethylase returned to normal (5 or 6 days). Although the acute hepatonecrotic action of DMN was only slightly reduced by CCl_4, the development of cytoplasmic basophilia occurred. The mechanism of the protective effect of CCl_4 requires elucidation.

0104 *IN VITRO* METABOLIC ACTIVATION OF CHEMICAL MUTAGENS. I. DEVELOPMENT OF AN *IN VITRO* MUTAGENICITY ASSAY USING LIVER MICROSOMAL ENZYMES FOR THE ACTIVATION OF DIMETHYLNITROSAMINE TO A MUTAGEN. (Eng.) Gletten, F. (Coll. Med., Howard Univ., Washington, D. C.); Weekes, U.; Brusick, D. *Mutat. Res.* 28(1):113-122; 1975.

Quantitative and qualitative assays were developed to study the *in vitro* enzymatic activation of dimethylnitrosamine to its mutagenic form. Three different fractions from mouse liver homogenates, including purified microsomes, were used for the activation. The reaction mixture contained the following components: Tris-HCl buffer, pH 7.4, 14.0 μM/ml; $MgCl_2$, 1.7 μM/ml; triphosphopyridine nucleotide, 5.9 μM/ml; isocitric acid, 49.0 μM/ml; and isocitric acid dehydrogenase, 1.0 U/ml. In qualitative plate tests, samples of the test compound and the bacterial indicator cells were added to the molten top agar to give final concentrations of 100 μM/ml and 2-5 x 10^8 cells/ml, resp. Following solidification, 0.1 ml samples of the reaction mixture were added to the center of the plate. All plates were incubated at 37 C for three days before scoring for revertants. In quantitative suspension tests, the isocitric acid dehydrogenase was added to the reaction mixture five min prior to starting the test followed by the addition of the bacterial indicator cells and the microsomal enzyme preparation. In the qualitative plate tests, hepatic enzyme preparations from six different mammals (human, monkey, rabbit, guinea pig, rat and mouse) were shown to possess the ability to generate mutagenic metabolites from dimethylnitrosamine. Enzyme fractions from animals of two inbred mouse strains (BALB/cJ and C57BL/6J) were compared in paired tests using the *in vitro* activation assay. This technique may be a useful tool in quantitatively measuring differences in genetically influenced levels of dimethylnitrosamine metabolism.

0105 CARCINOGENICITY OF METHYLATED DINITROSOPIPERAZINES IN RATS. (Eng.) Lijinsky, W. (Biol. Div., Oak Ridge Natl. Lab., Tenn.); Taylor, H. W. *Cancer Res.* 35(5):1270-1273; 1975.

Methylated dinitrosopiperazines were injected into 15 male and 15 female Sprague-Dawley rats to observe any detectable changes in the chemical structure of carcinogenic activities. Dinitrosopiperazines were prepared from amines by reaction with sodium nitrite in dilute acetic acid. Each group was given nitropiperazine followed by the administration of dinitrosopiperazine. Animals were submitted to complete necropsy, with tumors and lesions prepared for histological examination. The administration of methylated dinitrosopiperazine in rats revealed all compound-induced tumors in such areas as the nasal turbinates, upper gastrointestinal tract, liver and brain with nitrosamine being the apparent cause of death. Dinitrosopiperazine was shown to be the least potent carcinogen followed by 2,5-dimethyl derivative having slightly more potency. Dinitrosohomopropiperazine, 2-methyldinitrosopiperazine and 2,6-dimethyldinitrosopiperazine were found to be more effective in producing tumors. Smaller doses of the homologs led to more rapid death from tumors than those doses of the original compound. The lack of effect on carcinogenic activity of the methyl groups in 2,5-dimethyldinitrosopiperazine could be due to the effect of the other methyl group α to the nitroso function. Further studies are required to clarify this theory.

0106 COLON CANCER INDUCTION IN MICE BY INTRARECTAL INSTILLATION OF *N*-METHYLNITROSOUREA. (Eng.) Narisawa, T. (Naylor Dana Inst. Dis. Prev., New York, N. Y.); Weisburger, J. H. *Proc. Soc. Exp. Biol. Med.* 148(1):166-169; 1975.

The development of animal models of colon carcinogenesis by the intrarectal instillation of *N*-methylnitrosourea was examined. A 5.0 cm metal tube was inserted about half way into the lumen of the large intestine through the anal orifice of 7-8 week-old Swiss mice. In one group, mice were given 0.075 ml of a 2% solution of methylnitrosourea (1.5 mg) three times weekly for two weeks. In the second group, mice were given 0.075 ml of a 0.4% solution of methylnitrosourea (0.3 mg) three times weekly for ten weeks. In the control group, 0.075 ml distilled water was administered. Neoplasms of the large intestine were found in 14/30 mice (47%) in the first group and in 18/28 (64%) in the second group. The latent period was longer in the second group than in the first group. In the large intestine, adenocarcinomas and adenomas were found in the distal colon and rectum, and squamous cell carcinomas at the anal canal. Both adenocarcinomas and squamous cell carcinomas showed various types of invasion even to the outside of the intestinal wall in advanced cases. No metastases were found in the regional lymph nodes and other organs. All mice with large bowel neoplasms also had lung adenomas that were multiple small nodules; malignant lesions were not found. Leukemia was present in 10/14 mice with large bowel neoplasms in the first group and in 5/18 in the second group. It

is concluded that frequent applications of a small dose of methylnitrosourea gives better results in the production of colon cancer in mice by the intrarectal instillation procedure, compared with a less frequent application of a large dose.

0107 *N*-NITROSOMETHYLUREA AS MAMMARY GLAND CARCINOGEN IN RATS. (Eng.) Gullino, P. M. (Natl. Cancer Inst., Bethesda, Md.); Pettigrew, H. M.; Grantham, F. H. *J. Natl. Cancer Inst.* 54(2):401-414; 1975.

The efficacy of *N*-nitrosomethylurea (NMU) as a mammary gland carcinogen was studied in female BUF/N, F344, and Sprague-Dawley rats. Fifty-day-old animals were given three i.v. injections of NMU (5 mg/100 g) at four-week intervals. Other rats received NMU or 3-methylcholanthrene (MCA, 10 mg in 1 ml sesame oil/day for ten consecutive days) by stomach gavage. I.v. NMU produced mammary carcinomas in 89% of the BUF/N rats, 73% of the Sprague-Dawley rats, and 89% of the F344 rats, the latent periods averaging 77, 86, and 94 days, resp. Mortality among these animals was negligible. Castration prior to treatment prevented oncogenesis in the BUF/N rats; after treatment, it reduced the number of tumors/rat, but not the overall incidence of tumor-bearing animals. After the tumors were established, castration arrested the growth of the tumors or caused a temporary regression. Metastases to the bone marrow and spleen were consistently observed, with occasional metastases to the liver and lungs being found. After removal of the primary tumor, the metastases continued to grow, but at a slower rate than that observed in the primary tumor. Almost all tumors were transplantable i.p. and/or s.c. in the inguinal area of intact, ovariectomized, and adrenalectomized rats; transplanted tumors were able to metastasize. The doubling times of the primary and transplanted carcinomas were about seven days. Cachexia appeared during the fifth week after the onset of tumor and hypercalcemia was generally observed with tumors larger than 15 g. The NMU treatment program described appears to offer the best method available for inducing in rats a high yield of mammary carcinomas which closely mimic those found in humans, especially in postmenopausal women.

0108 DIFFERENTIAL ONCOGENIC EFFECTS OF METHYLNITROSOUREA. (Eng.) Swenberg, J. A. (Dep. Vet. Pathobiol., Ohio State Univ., Columbus); Koestner, A.; Wechsler, W.; Brunden, M.N.; Abe, H. *J. Natl. Cancer Inst.* 54(1):89-96; 1975.

Differences in the oncogenic effects of methylnitrosourea (MNU), induced by varying dose schedules and changing administration routes, were investigated. To elucidate tissue sensitivities, the interrelationships of dose scheduling and local *versus* resorptive carcinogenicity were evaluated. MNU was administered at 5 mg/kg/week *via* s.c., i.p., and p.o. routes for 36 weeks to groups of 23 to 26 rats beginning at nine weeks of age. Another series of 37 male rats received 180 or 360 mg/kg of MNU p.o. on a varying administration schedule. A third series of male rats received MNU i.v. at 25 mg/kg every four weeks for a total dose of 200 mg/kg. No uninoculated control animals were used in any of the experiments. The results showed that the central nervous system (CNS) was the target when MNU was given i.v. When given by other routes, there was a decrease in the number of neurogenic tumors and in the appearance of neoplasms at the injection site. Increased p.o. doses of MNU caused shorter survival times, a decreased incidence of neuroglial tumors, and increased numbers of thymic lymphomas and mesenchymal tumors of the CNS. The administration of MNU i.v. was intended to maximize the number of brain tumors and to minimize the number of injections. However, more animals developed extraneural and peripheral nervous system tumors than brain tumors. It is suggested that many tissues are susceptible to the oncogenic effects of MNU, but the degree of exposure necessary for neoplastic transformation varies.

0109 INDUCTION OF CARCINOMA OF THE LARGE INTESTINE IN GUINEA PIGS BY INTRARECTAL INSTILLATION OF *N*-METHYL-*N*-NITROSOUREA. (Eng.) Narisawa, T. (Naylor Dana Inst. Dis. Prev., New York, N.Y.); Wong, C. Q.; Weisburger, J. H. *J. Natl. Cancer Inst.* 54(3):785-787; 1975.

The induction of carcinomas of the large intestine in female inbred strain-2 guinea pigs after intrarectal instillation of *N*-methyl-*N*-nitrosourea (MNU) was investigated. MNU solutions were prepared in distilled water adjusted with Na_2HPO_4 and NaH_2PO_4 buffer solution to pH 6.5. A metal gastric feeding tube with a bulbous protective tip was inserted into the lumen of a large intestine through the anus before the solution was instilled. The solution filled the distal but did not enter the proximal large intestine. Sixteen experimental animals were given 0.5 ml MNU solution (1.25 mg MNU) twice weekly for 42 weeks; 16 controls were given distilled water adjusted to the same pH as the experimental group. Intrarectal instillation of MNU induced adenocarcinomas in the distal large intestine of these animals. The tumors were found to be constrictive or infiltrative. The intestinal wall in the area of the tumor was found to be thick and hard with a rough mucosal surface which had shallow ulcerations. The serosal surface was white due to tumor invasion to the serosa. No tumors were found in any animals of the control group. Facts presented in this study may provide the background for research on molecular aspects, diagnosis and treatment of human digestive cancer, particularly colon cancer.

0110 THE OCCURRENCE OF TUMOURS IN F_1, F_2 AND F_3 DESCENDANTS OF BD RATS EXPOSED TO N-NITROSOMETHYLUREA DURING PREGNANCY. (Eng.) Tomatis, L. (Int. Agency Res. Cancer, Lyons, France); Hilfrich, J.; Turusov, V. *Int. J. Cancer* 15(3):385-390; 1975.

The occurrence of tumors observed in F_1, F_2 and F_3 in descendants of BD rats exposed to N-nitrosemethylurea (NMU) during pregnancy was investigated. NMU in NaCl solution was administered i.p. to eight female BD-IV inbred rats in one dose of 20 mg/kg. The F_1, F_2, and F_3 progeny were mated on a brother-sis-

ter basis. Seven untreated pregnant females and their F_1 and F_2 progeny were used as controls. All rats were necropsied and the brain, liver, spleen, thymus, spine and gonads were fixed in buffered formalin (10%) and examined histologically. Tumors in F_1, F_2 and F_3 descendants of BD rats exposed to NMU exhibited no differences in survival rates between untreated control mothers and their F_1 and F_2 descendants and F_2 and F_3 descendants of NMU-treated mothers. Mortality rates were highest in mothers treated with NMU and their F_1 progeny. The percentage of tumor-bearing rats was higher in females treated with NMU during pregnancy and their F_1, F_2 and F_3 descendants as opposed to untreated females and their descendants. The most common tumors in descendants of NMU-treated mothers occurred in the nervous tissue (five) and in the kidney (six). Females of the F_2 generation had a higher incidence (43%) of mammary tumors when compared to that of the controls (13%). In the F_3 generation two tumors were observed in the nervous tissue of two male rats accompanied with a high incidence of meningiomas and a mammary tumor occurring in one male rat. No correlation could be drawn between the presence of tumors in F_1 and F_2 parents and the development of tumors in their descendants, F_2 and F_3. In untreated controls the most common tumors occurred in the mammary (10.3%) and pituitary (13.8%) glands. The results indicate that previous exposure to a carcinogen during pregnancy may result in an increased cancer risk which may persist through several generations.

0111 CELL CYCLE-DEPENDENT INDUCTION OF MUTATIONS ALONG A YEAST CHROMOSOME. (Eng.) Kee, S. G. (Dep. Biol., Brandeis Univ., Waltham, Mass.); Haber, J. E. *Proc. Natl. Acad. Sci. USA* 72(3):1179-1183; 1975.

DNA replication as it relates to the action of the mutagen *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) in *Saccharomyces cerevisiae* strain A31 (genotype *a leu1 trp*5 *cyh*2 *met*13 *tyr*3 *lys*5 *ade*5) was investigated. Six autotrophic markers along one arm of chromosome VII was used to study MNNG on a single yeast chromosome. Yeast was grown in liquid medium containing 1% yeast extract, 2% peptone, and 2% dextrose (YEPD). Zonal rotator separation was carried out by suspending cells in water and then loading the center of an Anderson zonal rotor containing sterile sucrose gradients. Exponetially growing cells of strain A31 were centrifuged, suspended in YEPD and treated with nitrosoguanidine followed by separation with a zonal rotor centrifuge into fractions equivalent to stages in the cell cycle. DNA content was measured colorimetrically with diphenylamine. The effect of MNNG during the cell cycle of *Saccharomyces cerevisae* revealed a 10-fold maximum increase in the frequency of prototroph than that found in other fractions of the cell cycle. Prototroph formation outside of DNA replication remained 10-fold higher than spontaneous frequency, suggesting that nitrosoguanidines may act in a second fashion independent of the stage of the cell cycle; this is thought to account for the lack of periodic induction of revertants of *met*13 and the broad effect of the mutagen on cell viability. Nitosoguanidine did not prove to act at or near the replication point in yeast. The frequencies of intragenic revertants of different markers along one arm of chromosome VII did not occur at the same time during DNA replication. The height of reversion frequency for *lys*5 was later than *leu*1 and *ade*5 markers, followed by *tyr*3 and *trp*5 occurring near the end of DNA replication. The results indicate that nitrosoquanidine acts as a mutagen during DNA replication, with different markers appearing to be affected at different intervals during the S period. This suggests that replication of one arm of chromosome VII requires more than a single point of initiation of DNA replication. Further investigation into the effect of DNA replication dependent on other cell functions is indicated.

0112 REDUCED DNA REPAIR CAPACITY AND INCREASED CYTOTOXICITY FOLLOWING SPLIT DOSES OF THE MUTAGEN 4-NITROQUINOLINE-I-OXIDE IN CULTURED HUMAN CELLS. (Eng.) Warren, P. M. (Cancer Res. Cent., Univ. British Columbia, Vancouver, Canada); Stich, H. F. *Mutat. Res.* 28(2):285-293, 1975.

Human fibroblasts were used to observe the reduction of DNA repairing capacities and increased cytotoxicity following split dosages of the mutagen 4-nitroquinoline-I-oxide (4NQO). Fibroblasts were obtained from a skin punch biopsy of a Caucasian female and maintained in Eagle's minimum essential medium (MEM) supplemented with fetal calf serum and antibiotics (penicillin, streptomycin, kanamycin and fungizone). DNA repair synthesis and semiconservative DNA replication was separated by culturing cells in media lacking arginine and fetal calf serum. DNA repair synthesis was estimated by allowing cells to undergo repair replication in the presence of tritiated thymidine ($[^3H]$ TdR) followed by exposure to 4NQO with estimation of silver grains over nuclei in autoradiographic preparations. Colony-forming capacity was determined by seeding cells and covering them with MEM supplemented with fetal calf serum with exposure to 4NQO. Chromosome aberrations were carried out by arresting dividing cells with colchicine. Reduction in DNA repair capacity and increased cytotoxicity after split dosages of 4NQO in human fibroblasts exhibited DNA repair synthesis occurring at different time intervals after 1-2 treatments with 4NQO. Application of 4NQO during a three hr period following the addition of the first treatment was found to be significantly low. Exposure of cells at a later period produced unscheduled $[^3H]$TdR incorporation which was comparable to that following a single treatment of 4NQO of equimolar concentrations. Chromosome aberrations were found to be elevated when the dose interval of 4NQO was given less than three hr apart. This investigation suggests that the reduced period of DNA repair capacity increases the mutagenic effect of the chemical carcinogen 4NQO.

0113 INDUCTION OF URINARY BLADDER TUMORS IN RATS BY ADMINISTRATION OF NITROSOMETHYLDODECYLAMINE. (Eng.) Lijinsky, W. (Biol. Div., Oak Ridge Natl. Lab., Tenn.); Taylor, H. W. *Cancer Res.* 35(4):958-961; 1975.

Nitrosomethyl-*n*-dodecylamine was prepared and admin-

istered to groups of 15 male and 15 female Sprague-Dawley rats to study the reaction of dimethyldodecylamine (a tertiary amine) and nitrous acid *in vivo*. Gavage administration was necessary because the compound's low solubility in water prevented feeding in drinking water. Twice weekly, 0.2 ml of a solution of 60 mg nitrosomethyl-*n*-dodecylamine/ml olive oil was administered by gastric intubation; this treatment began when the rats were 12 weeks old and was continued for 50 weeks. Nitrosomethyl-*n*-dodecylamine caused 100% incidence of transitional cell carcinomas of the urinary bladder. In contrast with other N-nitroso compounds, it was more efficient in males than females; females with tumors died later, although the dose per unit body weight was greater in females. The authors conclude that the highly uniform tumor response induced by this carcinogenic nitroso is unusual; the mechanism of action of nitrosomethyldodecylamine is worth investigation.

0114 INDUCTION OF ARYL HYDROCARBON HYDROXYLASE BY POLYCHLORINATED BIPHENYLS IN THE FOETO-PLACENTAL UNIT AND NEONATAL LIVERS DURING LACTATION. (Eng.) Alvares, A. P. (Rockefeller Univ., New York, N.Y.); Kappas, A. *FEBS Lett.* 50(2):172-174; 1975.

The effects of polychlorinated biphenyls (PCBs), phenobarbital (P), and 3-methylcholanthrene (3-MC) on the fetal environment and on neonatal liver enzymes were studied in Sprague-Dawley rats. Pregnant females were injected i.p. with 3-MC (25 mg/kg/day for 4 days), P (37.5 mg/kg twice daily for four days), or PCBs (25 mg/kg/day for six days) and sacrificed on the 20th day of pregnancy. In another series of experiments, similar treatments were instituted beginning on day two postpartum for the PCBs and on day four postpartum for P and 3-MC; the offspring of these females were sacrificed on day eight. The PCBs caused a 10-fold induction in the hydroxylase activity in the placentas of the pregnant rats, but only a 3-fold induction in the fetal livers. 3-MC, on the other hand, caused an 18- to 20-fold induction in both the placental and fetal liver hydroxylase activities. P had no significant effect on the placental hydroxylase activities but caused a 4-fold increase in the fetal liver hydroxylase activity. When administered to the lactating mothers, PCBs caused an 18-fold increase in the benzo(a)pyrene hydroxylase activity, a 3-fold increase in the cytochrome P-450 content, and a 2-fold increase in the N-demethylase activity of the neonatal liver. 3-MC treatment caused a significant decrease in the neonatal liver N-demethylase activity but did not affect cytochrome P-450 or the activity of N-demethylase. P treatment resulted in less than 2-fold increases in cytochrome P-450 and in the activities of N-demethylase and hydroxylase in the neonatal livers. The results suggest that the levels of N-demethylase, hydroxylase, and cytochrome P-450 may be altered in human infants exposed to PCBs through the maternal milk.

0115 THE COMPARATIVE CYTOGENETIC EFFECTS OF ALDRIN AND PHOSPHAMIDON. (Eng.) Georgian, L. (Victor Babes Inst. Pathol. Med. Genet., Bucharest, Romania). *Mutat. Res.* 31(2):103-108; 1975.

The cytogenetic effects of various doses of an organochlorinated pesticide (aldrin) and of an organophosphorus pesticide (phosphamidon) were examined *in vitro* and *in vivo*. In human lymphocyte cultures, aldrin produced chromosomal aberrations over the dose range 19.124-38.25 µg/ml. Phosphamidon was clastogenic at 1.9-122 µg/ml. With 61 and 35.5 µg/ml of phosphamidon, a large number of C-like mitoses were observed; lower concentrations produced a number of chromosome and chromatid rearrangements, as well as gaps, breaks, deletions, and fragments. Since cell death began at a phosphamidon concentration above 244 µg/ml, the chromosome aberrations, especially those induced by low doses, could be maintained in other abnormal cells. In the *in vivo* experiments, AKR mice and Wistar rats were injected i.p. with aldrin or phosphamidon, and chromosome examinations were performed 24 hr before harvesting the bone marrow. Minimal doses inducing chromosome aberrations were 9.56 µg/g of aldrin and 0.07 µg/g of phosphamidon. All interchanges and most of the deletions observed with phosphamidon were of the chromosome type, while most of the gaps, breaks, and fragments were of the chromatid type. Results of both *in vitro* and *in vivo* experiments showed a dose-response relation between the two pesticides and the number of aberrant cells and chromosome aberrations. It is concluded that low doses of pesticides with weak general toxicity could possibly damage somatic and gonadal cells.

0116 CYTOPLASMIC INCLUSIONS IN PULMONARY MACROPHAGES OF CIGARETTE SMOKERS. (Eng.) Brody, A. R. (Univ. Vermont Coll. Med., Burlington); Craighead, J. E. *J. Pathol.* 32(2):125-132; 1975.

Lung tissue from persons undergoing pneumonectomy or lobectomy for bronchogenic carcinoma and from patients with progressive idiopathic pulmonary fibrosis (25 cigarette smokers, six non-smokers) was examined by transmission electron microscopy and by energy-dispersive x-ray spectrometry. Lysosomes and phagolysosomes were prominent in the numerous pulmonary alveolar macrophages of cigarette smokers. Characteristic cytoplasmic structures, "smokers' inclusions", were seen by electron microscopy in the majority of these cells. The inclusions varied from 0.05-4 µm in length and were observed singly in relatively small phagolysosomes or stacked in aggregates in larger phagolysosomes. The inclusions also were present in scattered macrophages in the interstitium of the alveolar septa. Cross-sections of the structures were never seen in electron micrographs, indicating that a fiber or needle shape, as previously suggested, seems unlikely. Thin sectioning techniques imparted varying lengths to the inclusions, suggesting that they have a disc or platelike configuration. Smokers' inclusions were not present in the cells of biopsies from non-smokers. When lung tissue from smokers was digested in hot potassium hydroxide, varying numbers of hexagonal platelike particles were found. These particles had features consistent with those of the aluminum silicate kaolinite. Energy-dispersive x-ray spectrometry confirmed the presence of aluminum and silicon. Samples of tobacco from nine popular brands of cigarettes were digested in hot potassium silicate, yielding numerous hexa-

gonal platelike particles. Morphologic criteria, x-ray spectrometric analysis, and electron diffraction studies indicated that these particles were crystals of kaolinite. These results suggest that the aluminum silicate inclusions in pulmonary macrophages are derived from inhaled tobacco smoke.

0117 MALIGNANT TRANSFORMATION OF MOUSE CELLS BY CIGARETTE SMOKE CONDENSATE. (Eng.) Benedict, W. F. (Univ. Southern California Sch. Med., Los Angeles); Rucker, N.; Faust, J.; Kouri, R. E. *Cancer Res.* 35(3):857-860; 1975.

A low-nicotine cigarette smoke condensate, 12 fractions of the condensate, and a reconstituted sample were tested for their ability to induce transformation in the mouse cell line C3H/10T½CL8. Twelve 60-mm Petri dishes were seeded with 1000 cells in 5 ml medium for each sample to be tested. Twenty-four hr after seeding, test samples were added in 10-50 µliter aliquots. At five to six wk after treatment, the dishes were washed with distilled water, fixed with methanol, and stained with Giemsa. Dishes were scored for the presence of type III foci only; cells derived from type III foci have been shown to produce fibrosarcomas with 85-90% incidence when inoculated s.c. into irradiated C3H mice. Both the crude condensate and the reconstituted sample as well as two specific fractions induced transformation in the mouse cells. All transformed cell lines produced fibrosarcomas when 2×10^6 cells were injected into antithymocyte serum-treated syngeneic mice, whereas untreated control cells did not produce tumors. Cells injected into three-day-old mice gave tumors at a higher frequency and more rapidly than cells injected into six- to eight-wk-old mice. These results suggest that the 10T½CL8 cell line can be used for the detection and screening of potential oncogens and that using younger animals may be an efficacious method to test 10T½CL8 transformed cell lines for tumorigenicity.

0118 HISTOLOGIC TYPE OF LUNG CANCER IN RELATION TO SMOKING HABITS, YEAR OF DIAGNOSIS AND SITES OF METASTASES. (Eng.) Auerbach, O. (Veterans Adm. Hosp., East Orange, N.J.); Garfinkel, L.; Parks, V. R. *Chest* 67(4):382-387; 1975.

A study was made of histologic type of lung cancer in relation to smoking habit, year of diagnosis, and site and age of metastasis. Data on smoking habits was collected from the relatives of 662 men who had died during the period from 1955-1972. As classified by the World Health Organization system, 35.2% of the cancers were epidermoid carcinoma, 24.6% were small cell carcinoma, 25.2% were adenocarcinoma, and 14.2% were large cell undifferentiated carcinoma. The six nonsmokers of the study all had adenocarcinoma. A steady decrease in the incidence of small cell carcinoma was observed during this period, but it was not statistically significant. Small cell carcinoma increased with the amount of smoking, but not for all age groups. Adenocarcinomas decreased with advancing age, but not in all smoking groups. Metastases were found in 96.3% of the cases and the sites most frequently involved were regional lymph nodes (89.1%), liver (44.1%), brain (44.7%), adrenals (34%), distant lymph nodes (44.6%) and bone. Small cell carcinomas showed the greatest percentage of involvement for those major sites, and epidermoid carcinomas had the lowest percentage of metastases for the same major sites. No consistent trends by smoking habit, year of diagnosis, or age group were observed in this study.

0119 DNA SYNTHESIS IN CELL CULTURES FOLLOWING REPEATED EXPOSURE TO FRESH CIGARETTE SMOKE. (Eng.) Holt, P. G. (Univ. West. Australia, Perth Med. Cent., Shenton Park); Roberts, L. M.; Keast, D. *Experientia* 31(1):109-110; 1975.

The synthesis of ^{3}H-protein, ^{3}H-RNA, and ^{3}H-DNA was studied in murine peritoneal macrophages and murine embryonic fibroblasts exposed, in culture, to fresh cigarette smoke for up to two weeks. At low dosages (2-sec exposure to smoke mixed with air in a ratio of 1:14), macrophages showed stimulation of both ^{3}H-protein and ^{3}H-RNA synthesis. As the dosage increased, the degree of metabolic stimulation decreased, together with the viability of the cultures. Low smoke dosages had little effect on ^{3}H-DNA synthesis in macrophages, but higher dose regimes (two 2-sec exposures to a 1:7 mixture) produced a small stimulation (50%). At the end of one week, macrophage cultures showed an increasing proportion of fibroblast-like cells. The metabolic activity of fibroblast cultures showed little variability over a 1-week exposure, regardless of the smoke dosage used. Following a second week of exposure at the highest dose, these cells contained more viable cells than control cultures and ^{3}H-DNA synthesis rates were up to eight times higher than those of the controls. The data suggest that cigarette smoke may potentially exert dramatic effects on DNA synthesis in susceptible cells. Direct effects on nucleic acid synthesis may be involved in the development of hyperplastic changes in cells lining the respiratory tract of smokers, and may also play a part in the eventual development of neoplasia.

0120 THE CARCINOGENIC EFFECT OF 4,4'-METHYLENE-BIS-(2-CHLOROANILINE) IN MICE AND RATS. (Eng.) Russfield, A. B. (Bio-Res. Consult., Inc., Cambridge, Mass.); Homburger, F.; Boger, E.; Van Dongen, C. G.; Weisburger, E. K.; Weisburger, J. H. *Toxicol. Appl. Pharmacol.* 31(1):47-54; 1975.

0121 EXPERIMENTAL NEOPLASIA IN RATS FROM ORAL ADMINISTRATION OF 3,3'-DICHLOROBENZIDINE, 4,4'-METHYLENE-BIS(2-CHLOROANILINE), AND 4,4'-METHYLENE-BIS(2-METHYLANILINE). (Eng.) Stula, E. F. (Haskell Lab. Toxicol. Ind. Med., E. I. du Pont de Nemours Co., Inc., Wilmington, Del.); Sherman, H.; Zapp, J. A., Jr.; Clayton, J. W., Jr. *Toxicol. Appl. Pharmacol.* 31(1):159-176; 1975.

0122 ACTIVITY OF 6-METHYL-8-SUBSTITUTED ERGOLINES AGAINST THE 7,12-DIMETHYLBENZ[a]-ANTHRACENE-INDUCED MAMMARY CARCINOMA. (Eng.) Sweeney, M. J. (Lilly Res. Lab., Eli Lilly Co.,

Indianapolis, Ind.); Poore, G. A.; Kornfeld, E. C.; Bach, N. J.; Owen, N. V.; Clemens, J. A. *Cancer Res.* 35(1):106-109; 1975.

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See also:

* (Rev): 0001, 0002, 0003, 0004, 0005, 0006, 0018, 0021, 0022, 0023, 0024, 0025, 0026, 0027, 0028, 0029, 0030, 0031, 0032, 0033
* (Phys): 0195
* (Immun): 0294, 0322, 0323, 0344, 0345, 0366
* (Path): 0397, 0448, 0459, 0461, 0462, 0464, 0493, 0497
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0179 POST-IRRADIATION PROLIFERATION KINETICS OF A SERIALLY TRANSPLANTED MURINE ADENOCARCINOMA. (Eng.) Szczepanski, L. v. (Institut fur Biologie der Gesellschaft fur Strahlen- und Umweltforschung, 8042 Neuherberg, West Germany); Trott, K.-R. *Br. J. Radiol.* 48(567):200-208; 1975.

The proliferation pattern of a mouse mammary carcinoma was investigated during the first week after single exposures of 600 and 1200 rads in a study of optimal dose-fractionation schedules in radiotherapy. The experiments were performed on the murine adenocarcinoma 284, growing in female C3H mice (10-12 weeks old). The tumor was serially transplanted by s.c. implantation of 2 mm pieces into the right scapular region. The animals were anesthetized with hexobarbital before the tumor was exposed to 300 kV x rays, at an exposure rate of 60 rad/min. Cell-cycle parameters were studied by counting the ratio of labeled to total mitoses at various times after a single injection of 30 μCi of ^{3}H-thymidine i.p. Animals were sacrificed every 30 min during the first 12 hr after labeling. Every hr up to 36 hr after labeling, the tumors were removed and subjected to autoradiography. One hundred mitoses were counted in each tumor to define the percentage of labeled mitoses. The earliest effect of irradiation was synchronization of the proliferating cells of the tumors. The tumors regressed rapidly and after 48 hr were only 15-20% of their preirradiation sizes. Regrowth started six days after a dose of 600 rads but was not measurable before day eight after a dose of 1200 rads. Minor changes in the cell-cycle times were found within the first week after irradiation. There were considerable fluctuations in the mitotic index after irradiation. The labeling index rose after repeated ^{3}H-thymidine injections were given at various times after a dose of 600 rads. This rise in the labeling index was due to: the inflow of unlabeled cells into S-phase; mitotic division of the labeled cells; and elimination of unlabeled cells from the tumor. It is concluded that, depending on the dose, resting cells are triggered, three or four days after irradiation, to increase the growth fraction. The nature of this trigger remains obscure. It is also suggested that the triggering of G_0 cells into the cycle by irradiation may be one mechanism rendering fractionated radiotherapy more effective than single large doses of radiation.

0180 THYMIC REGENERATION AFTER LETHAL IRRADIATION: EVIDENCE FOR AN INTRA-THYMIC RADIORESISTANT T CELL PRECURSOR. (Eng.) Kadish, J. L.; (New York Univ. Sch. Med., N. Y.); Basch, R. S. *J. Immunol* 114(1/Part 2):452-458; 1975.

The effects of lethal irradiation on thymic regeneration in female A/J mice (12-20 weeks old) were investigated in an attempt to define the stem cell relationships of this differentiating system. Post-irradiation thymic and splenic regeneration were quantified by measuring the incorporation of ^{3}H-thymidine at various times after two different x-ray doses (760 or 890 rads). At each dose an attempt was made to influence the rate or extent of regeneration by transfusing syngeneic bone marrow cells. The thymus and spleen were removed two hr after i.v. injection of 10 μCi of ^{3}H-thymidine and radioactivity was measured by liquid scintillation spectrometry. Thymic regeneration was compared in mice receiving whole body lethal x-irradiation and those whose lower body was shielded. No significant difference was seen between the thymidine incorporation of bone marrow-protected and control animals during the first 12 days after irradiation. The amount of thymic regeneration was extensive, especially in the animals receiving 760 rads. ^{3}H-thymidine incorporation by the thymus began 48-72 hr after irradiation and increased throughout the next eight days. By the ninth day after 760 rads, typical corticomedullary architecture had been restored. The amount of thymic regeneration in the 890 rad group was only a fraction of that found at the lower radiation dose; the peak average thymidine incorporation (day seven) was 22 times that found on day one. Splenic regeneration was suppressed by both doses of radiation. The x-ray dose-response of thymic regeneration indicated that, even at an x-ray dose six times the LD_{90} of the bulk of the population, there remained a cell type capable of supporting mitotic activity in the thymus eight days after x-irradiation. The cells repopulating the thymus were morphologically indistinguishable from normal thymocytes and were susceptible to cytotoxic antisera against the thymic differentiation antigens Thy-1, TL, LyA_2 and LyC_2. It is concluded that the thymic regeneration seen after lethal x-irradiation demonstrates the existence of a previously unrecognized stem cell pool having substantial radioresistance.

0181 PLOIDY LEVEL AND MUTATION TO HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL-TRANSFERASE (HGPRT) DEFICIENCY IN CHINESE HAMSTER CELLS. (Eng.) Van Zeeland, A. A. (Dep. Radiat. Genet. Chem. Mutagenesis, State Univ. Leiden, Netherlands); Simon, J. W. I. M. *Mutat. Res.* 28(2):239-250; 1975.

In order to investigate the number of chromosomes bearing the hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) gene, the frequencies of X-ray-induced mutation of resistance to 8-azaguanine (AG) were measured in Chinese hamster cell lines; spontaneous and induced mutants for glucose-6-phosphate dehydrogenase (G6PD) and HGPRT activity were also analyzed. Two sets of matched cell lines, DON-2n and DON-4n, and V79-2n and V79-4n, were cultured; in selection experiments, AG (20 μg/ml) was added immediately before use. The cells were irradiated at 117 R/min (100 kV, 3 mA). The radiation-induced mutation frequencies in DON-2n and DON-4n were nearly identical. They differed by a factor of 44 for V79-2n and V79-4n, implying a chromosome loss in the tetraploid mutant line. No differences in G6PD activity of spontaneous and induced mutants were found in the near diploid lines or in the DON-4n line. The AG-resistant mutants derived from DON-2n, DON-4n, and V79-2n had essentially no HGPRT activity, while V79-4n mutants retained 50-100% activity. The V79-4n mutants were found uniform in growth and nonheterogeneous in G6PD activity; cross-resistance to 6-thiaguanine (20 μg/ml) was poor. Thus, mutations to AG resistance were not equally efficiently induced in near diploid and near tetraploid cell lines. Most of the

DON-4n mutants had an equal or higher G6PD activity than the parental lines, and apparently did not lose a chromosome bearing the HGPRT-gene; induced V79-4n mutants could be accounted to chromosome loss in accordance with a recessive mutation. The results obtained with the different cell line (with different ploidy levels) cannot be explained by a single genetic mechanism.

0182 RADIATION-INDUCED CHROMOSOME ABERRATIONS IN HUMAN PERIPHERAL LYMPHOCYTES. EXPOSURE TO X-RAYS OR PROTONS. (Eng.) Todorov, S. L. (Med. Acad., Sofia, Bulgaria). *Strahlentherapie* 149(2): 197-204; 1975.

A quantitative characterization of chromosome response to 180 kV X-ray and to 50 MeV protons in human peripheral lymphocytes was made. Doses used for the X-ray experiments were 25, 50, 100, 200, 250, 400, and 500 rads given at 1.13 rads/sec; exposure to protons occurred at 25, 50, 100, 250, and 500 rads delivered at 0.62 rads/sec. Analysis of structural chromosome aberrations indicated spontaneous rates to be low in human lymphocytes. In the X-ray experiments nonirradiated metaphases showed 0.016 aberrations per cell; the rate in proton experiments was 0.026 aberrations per cell. The rate for all types of chromosome anomaly, except for chromatid fragments, rose with increased dose. Among all types of anomalies observed in the X-ray experiment, chromosome fragments constituted 30-45% and dicentrics were 20-40%. Dicentrics were the most frequently seen type of two-break aberration (56-66%). A comparison of one- and two-break aberrations showed one-break aberrations to exceed by a factor of four at 25 rads. At 200 rads the rates were equal, and by 500 rads the proportions were reversed, with twice as many two- as one-break aberrations. A similar picture was obtained in the proton experiments, where chromosome fragments constituted 40-50% of the observed anomalies. Dicentrics were the most numerous (35-45%) two-break aberrations in doses up to 100 rads, increasing to 55-65% for larger doses. At 25 rads the number of one-break aberrations was twice that of two-break ones. At 50 and 100 rads the numbers were equal, and by 250 rads there were 1.5 times more two- than one-break aberrations. Chromosome fragments and aberrant cells were found in both series to have linear dose-response kinetics, while two-break aberrations, total aberrations, and total breakage showed nonlinearity. The data for both radiation types was found to fit two mathematical models: (1) the total number of aberrations, total number of breaks, and two-break aberrations fit a second-degree polynomial, $Y = bD + cD^2$, and (2) the model $Y = a + bD$ fits one-break aberrations and the number of aberrant cells.

0183 AGE-DEPENDENT EXCISION REPAIR OF DAMAGED THYMINE FROM γ-IRRADIATED DNA BY ISOLATED NUCLEI FROM HUMAN FIBROBLASTS. (Eng.) Mattern, M. R. (Dep. Biochem., Univ. Florida, Gainesville); Cerutti, P. A. *Nature* 254(5499):450-452; 1975.

The capacity of isolated nuclei and nuclear sonicates from young (15-25 passages), medium-aged (26-42 passages), and old (over 42 passages) diploid human lung fibroblast WI-38 cells to excise damaged thymine residues of the 5,6-dihydroxydihydrothymine type (t') from exogenous γ-irradiated or osmium tetroxide (OsO_4-oxidized DNA substrates was examined. Young and medium-aged nuclei removed approximately 1/4 - 1/3 of t' from the γ-irradiated acid-precipitable PM2 DNA within 60 min of incubation at 37 C; no excision of t' was accomplished by old nuclei. Only 2-6% of total thymine label was rendered acid-soluble during 60 min incubation. Excision of t' nuclei from young and medium-aged cells remained incomplete. Analogous data were obtained for the excision of t' from γ-irradiated PM2 DNA after incubation with nuclear sonicates. The capacity of WI-38 nuclei to excise t' from OsO_4-oxidized polyd(A-T) also depended on cell age. Nuclei from young cells selectively excised 28% of t', while 22% was excised by medium-aged cells. No excision was accomplished by old cells. The excision of t' from OsO_4-oxidized polyd(A-T) by young and medium-aged nuclei occurs with very high selectivity, the acid solubilization of thymine label being only 0.6-1%. Isolated nuclei or nuclear sonicates from senescent diploid human lung fibroblasts WI-38 have apparently lost their ability to excise, from DNA, thymine damaged by γ-rays. The fact that excision occurred from OsO_4-oxidized polyd indicates that neither damage to bases other than thymine nor radiation induced strand breakage is required for removal of t' from DNA. Finally, the extent of polymer degradation accompanying excision is small.

0184 RELATIVE GENETIC EFFICIENCY OF 50 MeV PROTONS AS DERIVED FROM ANALYSES OF CHROMOSOME ANOMALIES IN HUMAN LYMPHOCYTES. (Eng.) Todorov, S. L. (Med. Acad., Sofia, Bulgaria). *Strahlentherapie* 149(2):188-193; 1975.

The relative genetic efficiency (RGE) of 50 MeV protons was examined on the basis of quantitative characteristics of chromosome aberration induction in human peripheral lymphocytes. RGE values were calculated by dividing the 180 kV X-ray dose producing a fixed number of aberrations by the 50 MeV proton dose producing the same number of aberrations. Based on dicentric production by radiation doses ranging from 70 to 700 rads, RGE values ranged from 0.81-0.92. Similar values were obtained for minutes (0.70-0.93). A lower than unity coefficient is suggested, implying that the efficiency of protons in producing exchange-type aberrations is lower relative to X-rays. The chromosome fragment yields for 50 MeV proton RGE ranged from 1.10 to 1.16, suggesting higher efficiency of protons relative to X-rays. Based on the total number of aberrations and total breakage, the mean RGE of 50 MeV protons was 1.06 and 1.09, resp. At the cellular level, radiation-induced chromosome anomalies are an appropriate criterion for estimating the RGE of radiations differing in quality.

0185 SALIVARY GLAND NEOPLASMS FOLLOWING ATOMIC RADIATION: ADDITIONAL CASES AND REANALYSIS OF COMBINED DATA IN A FIXED POPULATION, 1957-1970.

(Eng.) Belsky, J. L. (At. Bomb Casualty Comm., Hiroshima, Japan); Takeichi, N.; Yamamoto, T.; Cihak, R. W.; Hirose, F.; Ezaki, H.; Inoue, S.; Blot, W. J. *Cancer* 35(2):555-559; 1975.

Eight additional cases of salivary gland tumors among atomic bomb survivors are reported, bringing the total to 30 cases. Of the new cases, only one, an adenocarcinoma, was considered malignant; six cases were mixed tumors and one was a papillary cystadenoma. There were no distinguishing histologic aspects among tumors in irradiated subjects that differed from those in nonexposed persons. The number of cases in the high (300+ rads) radiation dose group was significantly greater ($p < 0.01$) than would be expected if the 30 tumors were distributed among the dose groups in proportion to person-year (1957-1970). This relationship is also true for the nine malignant cases, but not for histologically benign tumors. The relative risks for total cases and for malignant growths are 9.35 and 21.8, resp. The authors' previous conclusion that younger persons were at significantly greater risk is weakened by this new analysis.

0186 ODONTOMA FORMATION IN THE JAW FOLLOWING IRRADIATION OF AN ADOLESCENT. (Eng.) Ostrowski, M. J. (Queen Elizabeth Hosp., Birmingham, England). *Br. J. Radiol.* 47(564):897-900; 1974.

0187 SYNERGISM BETWEEN DIFFERENT NEAR-ULTRA-VIOLET WAVELENGTHS IN THE INACTIVATION OF TRANSFORMING DNA. (Eng.) Peak, M. J. (Div. Biol. Med. Res., Argonne Natl. Lab., Ill.); Peak, J. G.; Webb, R. B. *Photochem. Photobiol.* 21(2):129-131; 1975.

0188 REPAIR OF DAMAGE INDUCED BY ULTRAVIOLET RADIATION IN MUTATOR T-1 *ESCHERICHIA COLI* TRANSDUCTANTS. (Eng.) Sideropoulos, A. S. (Med. Coll. Pennsylvania, Philadelphia); Greenberg, J.; Warren, G. *J. Am. Med. Wom. Assoc.* 30(2):65-67, 70-71; 1975.

0189 ON THE MECHANISM OF PRODUCTION OF CHROMOSOMAL ABERRATIONS BY ULTRAVIOLET RADIATION. (Eng.) Luchnik, N. V. (Inst. Med. Radiol., Acad. Med. Sci. USSR, Obninsk). *Mutat. Res.* 27(2):295-298; 1975.

0190 ULTRASTRUCTURAL DEMONSTRATION OF PECULIAR LYMPHOID CELLS IN THE THYMUS DURING THE PERINATAL PERIOD IN MICE. CORRELATION WITH RADIO-LEUKEMOGENESIS. (Eng.) Carpentier, J. L. (Lab. Anat. Pathol., Univ. Liege, Belgium); Boniver, J.; Betz, E. H.; Simar, L. J. *Eur. J. Cancer* 11(1): 65-69; 1975.

0191 THE YIELD OF CHROMOSOMAL ABERRATIONS IN RABBIT LYMPHOCYTES AFTER IRRADIATION *IN VITRO* AND *IN VIVO*. (Eng.) Bajerska, A. (Inst. Occup. Med., Sch. Med., Lodz, Poland); Liniecki, J. *Mutat. Res.* 27(2):271-284; 1975.

0192 EXPEDIENCE AND EXPERIENCE WITH REFERENCE TO CARCINOMA OF THE BRONCHUS. (Eng.) Backhouse, T. W. (Coventry and Warwickshire Hosp., Coventry, England). *Proc. R. Soc. Med.* 68(2): 115-120; 1975.

0193 CANCER IN THE IRRADIATED THYROID. (Eng.) Fisher, J. N. (Univ. Tennessee, Memphis); Frankel, K. A.; Bordin, G. M.; Refetoff, S.; Brewin, T. B.; Van Herle, A. J.; Uller, R. P.; Braverman, L. E.; Masse, F. *N. Engl. J. Med.* 292(18):975-977; 1975.

0194 INDUCED RADIATION RESISTANCE IN CELL CULTURES OF CHINESE HAMSTER CELLS: INDUCTION AND MANNER OF RESISTANCE. (Ger.) Keusch, F. (Strahlenbiologisches Institut der Universitat Zurich, August-Forel-Strasse 7, CH-8029 Zurich, Switzerland); Riehle, I.; Fritz-Niggli, H. *Experientia* 31(7):844-846; 1975.

0195 ASSOCIATION OF ASBESTOS AND BRONCHOGENIC CARCINOMA IN A POPULATION WITH LOW ASBESTOS EXPOSURE. (Eng.) Warnock, M. L. (Pritzker Sch. Med., Univ. Chicago, Ill.); Churg, A. M. *Cancer* 35(4):1236-1242; 1975.

See also:

* (Rev): 0017, 0018, 0031
* (Chem): 0067, 0091
* (Path): 0401, 0431, 0496

0196 NUCLEIC ACIDS OF RNA TUMOR VIRUSES: IDENTIFICATION AND ULTRASTRUCTURE. (Eng.)
Weber, G. H. (Natl. Cancer Inst., Bethesda, Md.); Heine, U.; Cottler-Fox, M. *Virology* 64(1):205-216; 1975.

Nucleic acids from both MC26 myelocytomatosis virus and Rous sarcoma virus, Prague strain(RSV-Pr), were spread with the Kleinschmidt technique and examined with the electron microscope. MC29 virus, RSV-Pr, and vesicular stomatitis virus (VSV) were purified and primary cultures of chick embryo cells were prepared and infected with strain MC29 virus. The viral RNA was isolated and studied *via* sedimentation analysis, nuclease denaturation and electron microscopy, employing aqueous spreading, formamide spreading, and urea-formamide spreading. Examination of the 60-70S RNA from MC29 and RSV-Pr virions revealed that the molecules were extended only when spread in the presence of 4 M urea and 80% formamide; an absence of significant differences in the sedimentation behavior of both RNAs was noted. Electron microscopic examination of the RNA isolated from MC29 and RSV-Pr viruses revealed, under certain conditions, the presence of long, smoothly contoured molecules; it appeared that the samples of high molecular weight viral RNA and/or unfractionated viral RNA contained a mixture of both DNA and RNA molecules in which DNA represented only a very small fraction of the complete sample. The results suggest that the observed distribution of RNA molecule-lengths of each species represent undegraded viral RNA subunits, suggesting redundancy and uniqueness present within each genome. The unexpected presence of a small number of DNA molecules in the preparations of the 60-70S viral RNA oncornaviruses, as revealed by the Kleinschmidt technique and confirmed by selective denaturation, suggests that the molecular lengths of the DNA strands observed were formerly assumed to represent the 60-70S RNA.

0197 INHIBITION OF REVERSE TRANSCRIPTASE ACTIVITY OF RNA-TUMOR VIRUSES BY FAGARONINE.
(Eng.) Sethi, V. S. (Litton Bionetics, Inc., Kensington, Md.); Sethi, M. L. *Biochem. Biophys. Res. Commun.* 63(4):1070-1076; 1975.

The inhibition of reverse transcriptase activity from murine leukemia virus (MuLV), simian sarcoma virus (SSV-1) and avian myeloblastosis virus (AMV) at increasing concentrations of fagaronine was examined. In a standard assay mixture of 0.10 ml, containing 10 µl of MuLV, 5 µl of SSV-1, or 5 µl of AMV, DNA polymerase concentrations of 0.50 µg/ml of the alkaloid were used for enzyme inhibition. There was a sharp decline in enzyme activity at increasing concentrations of the alkaloid, and inhibition curves were very similar to each other. The amount of alkaloid required to cause 50% enzyme inhibition was in the range of 6-12 µg/ml of reaction mixture. The enzyme activity with poly rA x oligo dT, poly dA x oligo dT, activated DNA and 70S viral RNA was strongly inhibited by the alkaloid. With poly rC x oligo dG there was no enzyme inhibition. When poly rA/oligo dT concentration in the assay mixture was increased from 2-17 µg, the enzyme activity was restored to about 60%, whereas a 4-fold increase of enzyme concentration completely reversed the inhibition. Fagaronine was added to the reaction mixture before incubation at 37 C and inhibitor effects were directed to free template primer and its complexes with enzyme substrate and metal ions. It is concluded that fagaronine inhibits oncornavirus DNA polymerase by interacting with (rA)n, (dT)n, or A:T pairs. However, it is yet to be determined whether fagaronine's anti-leukemic activity can be attributed to this property.

0198 NEOPLASTIC DIFFERENTIATION: INTERACTION OF SIMIAN VIRUS 40 AND POLYOMA VIRUS WITH MURINE TERATOCARCINOMA CELLS *IN VITRO*. (Eng.)
Swartzendruber, D. E. (Univ. Colorado Med. Cent., Denver); Lehman, J. M. *J. Cell. Physiol.* 85(2): 179-188; 1975.

The host-virus interactions of simian virus 40 (SV40) and polyoma virus (Py) with the multipotential stem cell of the teratocarcinoma, embryonal carcinoma and differentiated cells derived from embryonal carcinoma were investigated. When colonies of embryonal carcinoma were allowed to spontaneously differentiate for four or five days, cultures containing embryonal carcinoma, parietal yolk sac and spindle cells developed. Eight separate cultures of pure embryonal carcinoma were infected with SV40 virus either in single cell suspension or as small colonies at multiplicities of 0.1, 1, 10, 100 and 1000 plaque-forming units (PFU)/cell. The cells were assayed for the presence of T and V antigens at 24, 48, 72 and 120 hr after infection, and at no time could either antigen be detected by immunofluorescent staining. Embryonal carcinoma cells at ten different passage levels in tissue culture were used for infection with SV40 and each was resistant to infection. Infection of permissive cells induced the production of both T and V antigens. Twelve mixed teratocarcinoma cultures containing predominantly differentiated cells (parietal yolk sac and spindle cells) and some undifferentiated embryonal carcinoma cells were infected with SV40 virus at a multiplicity of 10 PFU/cell. By 48 hr about 95% of the cells contained intranuclear T antigen. When four replicate cultures of differentiated cells were infected with SV40, 100% of the cells became T antigen positive. The four parental strains of differentiated cells grew to confluency very slowly and exhibited contact inhibition of growth. The SV40 infected cells grew very rapidly, piled up after reaching confluency and have remained 100% T antigen positive for over six months. These results show that embryonal carcinoma cells, the stem cells of the teratocarcinoma, are apparently resistant to SV40 infection (at the level of T antigen expression) whereas their benign and more differentiated progeny are susceptible to virus infection in a typical non-permissive manner.

0199 STRUCTURAL PROTEINS OF A HUMAN PAPOVAVIRUS (BK VIRUS): A COMPARISON WITH THE STRUCTURAL PROTEINS OF SIMIAN VIRUS 40. (Eng.) Barbanti-Brodano, G. (Inst. Microbiol., Univ. Bologna, Italy); Minelli, G. P.; Portolani, M.; Lambertini, L.; Toppini, M. *Virology* 64(1):269-271; 1975.

The protein composition of simian virus 40 (SV40) and human papovavirus (BK virus) was analyzed by polyacrylamide gel electrophoresis. SV40 (strain Rh911) and BK virus were grown in Vero cells, concentrated, and purified; full particles of both viruses were separated and used throughout. Analyzation of purified SV40 by polyacrylamide gel electrophoresis revealed one major and four minor proteins. The same analysis of purified BK virus revealed six proteins; the major component, plus two minor components had a lower molecular weight than the comparable SV40 components. Another protein of molecular weight 34,500, absent in SV40, was detected migrating closely to VP2 (MW 36,500) in BK virus. The two faster-migrating polypeptides, shown to be of cellular origin in SV40, had the same electrophoretic ability in SV40 and BK virus. The data illustrated that the different virion polypeptides are present in approximately equal amounts in the SV40 and BK virus. The data suggest that SV40 polypeptides coded by the viral genomes are different in electrophoretic mobility, and presumably also in structure and composition, from the corresponding BK virus polypeptides, whereas viral polypeptides originating from the host cell are very similar in the two viruses.

0200 DISTRIBUTION OF MEMBRANE PARTICLES AND GAP JUNCTIONS IN NORMAL AND TRANSFORMED 3T3 CELLS STUDIED *IN SITU*, IN SUSPENSION, AND TREATED WITH CONCANAVALIN A. (Eng.) Pinto da Silva, P. (Salk Inst. Biol. Stud., San Diego, Calif.); Martinez-Palomo, A. *Proc. Natl. Acad. Sci. USA* 72(2):572-576; 1975.

Balb/c and Swiss 3T3 fibroblasts, normal and transformed by simian virus 40 (SV40) or murine sarcoma virus (MSV), were used to study membrane particle distribution. Some cell cultures were fixed *in situ* and incubated in phosphate-buffered saline with or without concanavalin A. Other cultures were fixed after being put in suspension by EDTA and incubated with or without concanavalin A. Cells were impregnated with glycerol after fixation, frozen in Freon 22 and freeze-fractured. Transformed and untransformed Balb/c and 3T3 cells, when fixed *in situ* and freeze-fractured, both showed a random distribution of membrane particles. This was true of subconfluent and confluent cultures, even after ten days of confluency. The only particle clusters were identified as gap junctions. These were more common in the SV40-transformed than in the untransformed Balb/c cells. Treatment of either cell type with concanavalin A had no effect. Cells fixed in suspension also had randomly distributed membrane particles. If cells were incubated in phosphate-buffered saline at 37 C for 30 min, up to 50% of the cells showed aggregation of the particles. In Balb/c cells aggregation was more frequent, and more intense in those transformed by SV40. In SV40-transformed and normal 3T3 cells, aggregation occurred in about 10%. In membranes with clear particle aggregation, the entire particle population congregated into a network. Exposure of unfixed Balb/c cells to glycerol resulted in a similar but less clear pattern. Treatment of cell suspensions with concanavalin A did not alter patterns relative to controls. It is concluded that the freeze-fracture morphology of the plasma membranes of normal and transformed cells have a basic similarity, and that concanavalin molecules have no specific effect on normal or transformed cells in suspension.

0201 CONTACT INHIBITION OF PHAGOCYTOSIS IN EPITHELIAL SHEETS: ALTERATIONS OF CELL SURFACE PROPERTIES INDUCED BY CELL-CELL CONTACTS. (Eng.) Vasiliev, J. M. (Acad. Med. Sci. USSR, Moscow); Gelfand, I. M.; Domnina, L. V.; Zacharova, O. S.; Ljubimov, A. V. *Proc. Natl. Acad. Sci. USA* 72(2):719-722; 1975.

Contact inhibition of phagocytosis was studied in five cell types. In epithelial sheets formed by primary explants of mouse kidney, transformed MPTR mouse kidney, and differentiated mouse hepatoma 60, it was found that central cells, which form firm intercellular contacts, exhibited low phagocytosis; marginal cells in the same sheets demonstrated greater phagocytic activity. After incubation for 6-2 hr in a medium to which carmine suspension (0.01-0.03%) had been added, the mean number of particles/marginal MPTR cell was 6-10 times greater than that per central cell. In dense cultures of trypsinized mouse embryo fibroblasts and of anaplastic mouse hepatoma 22a, no difference in phagocytosis between marginal and central cells was discovered. The upper surface of epithelial sheets was found to be nonadhesive for both prelabeled epithelial cells and fibroblasts, while the upper surface of dense cultures of mouse fibroblasts was adhesive for these cells. Anaplastic mouse hepatoma 22a showed higher adhesiveness than the epithelial sheets, but less than the fibroblasts. Agents inhibiting phagocytosis and also the movement of the cells included cytochalasin B, sodium azide, sorbitol, and incubation at low temperature (4 C). Movement at the free edge, the continuous formation of lamellipodia and ruffles, seemed to be a necessary condition of adhesiveness, hence of phagocytosis. It is suggested that contact inhibition of phagocytosis results from differential adhesiveness of the upper cell surface and of surfaces near the free edge. The formation of firm intercellular contacts appears to be sufficient to prevent ruffling at the contacting edges and to inhibit phagocytosis.

0202 INTEGRATION OF VIRAL GENOMES. (Eng.) Zhadnov, V. M. (D. I. Ivanovsky Inst. Virology, Moscow, U.S.S.R.). *Nature* 256(5517):471-473; 1975.

Transcription of virus RNA into DNA was studied in three chronically infected tissue culture systems: chick embryo fibroblasts infected with measles virus (MV), mouse L cells infected with Sindbis virus (SV), and human Hep-2 cells infected with tick-borne encephalitis virus (TBEV). Actinomycin D inhibited the synthesis of virus-specific structures in chronically infected cells but not in acutely infected cells. DNA from chronically infected cells contained sequences homologous to RNA of the corresponding viruses, whereas no such sequences could be found in noninfected cells. TBEV was isolated from swine cells treated with DNA from TBEV-infected Hep-2 cells, and SV was

isolated from the brains of mice inoculated with DNA from SV-infected L-cells. In both cases, DNase destroyed the infectivity of the DNA preparations, but RNase had no effect. The possibility that the transcription of virus RNA into double-stranded DNA was accomplished by latent oncornaviruses in culture was investigated using a test that reveals reverse transcriptase activity associated with high-molecular weight (60-70S) RNA. This activity was shown in all three chronically infected tissue cultures and also in uninfected L and Hep-2 cells. These results suggest an interaction between infectious viruses and latent oncornaviruses resulting in the transcription of the infectious virus RNA genomes into double-stranded DNA, which is then integrated into the genomes of the chronically infected cell. DNA sequences homologous to MV were also found in tissues and WBC of patients with systemic lupus erythematosus. The reverse transcriptase assay was positive with cytoplasmic extract from lupus erythematosus tissues, and parallel studies showed the presence of tubular structures in lupus-affected tissues and of MV antigens in WBC. A possible molecular mechanism for the initial events in the development of systemic lupus erythematosus is outlined.

0203 METHYLATION-DEPENDENT TRANSLATION OF VIRAL MESSENGER RNAs *IN VITRO*. *(Eng.)* Both, G. W. (Roche Inst. Mol. Biol., Nutley, N.J.); Banerjee, A, K.; Shatkin, A. J. *Proc. Natl. Acad. Sci. USA* 72(3):1189-1193; 1975.

Methylated and unmethylated RNAs synthesized *in vitro* by purified reovirus (type 3 Dearing strain) and vesicular stomatitis virus (VSV) (Indiana serotype) were compared as messengers in a cell-free protein-synthesizing system prepared from wheat germ. Methylated VSV and reovirus mRNA were synthesized in the presence of S-adenosylmethionine and the methylated and unmethylated mRNAs were incubated with wheat germ extracts. The methylated mRNAs stimulated protein synthesis by these extracts to a greater extent than the unmethylated mRNAs. The addition of S-adenosylmethionine to a cell-free extract programmed with unmethylated mRNA resulted in methylation of the mRNA and stimulated protein synthesis, while S-adenosylhomocysteine, an inhibitior of mRNA methylation, blocked the translation of the unmethylated, but not of the pre-methylated mRNAs, and inhibited protein synthesis by more than 95%. Aurintricarboxylic acid, which inhibits polypeptide chain initiation, also prevented mRNA methylation by wheat germ extracts, whereas sparsomycin, which inhibits polypeptide chain elongation, did not reduce mRNA methylation. The data indicate that in extracts of wheat germ, cell-free protein synthesis directed by VSV and reovirus mRNA is dependent on the methylation of the viral mRNAs. They also suggest that the methylation of viral mRNA in these extracts is dependent upon the initiation of protein synthesis.

0204 RETENTION OF TUMOUR-INDUCING CAPACITY BY ADENOVIRUS DNA AFTER CLEAVAGE BY RESTRICTION ENDONUCLEASES. (Eng.) Burnett, J. P. (Lilly Res. Lab., Div. Eli Lilly Co., Indianapolis, Indiana); Mayne, N.; Helton, L. *Nature* 254(5496):158-159; 1975.

The effect of several endonucleases on the oncogenic activity of Simian adenovirus SA7 DNA was studied. DNA was extracted using a phenol method. Restriction endonucleases produce defined fragments of the SA7 genome. The endonucleases which were purified and utilized included *Eco*RI, *Eco*RII, *Hind*, *Hga*, *Hpa*I, and *Hpa*II. The number of fragments produced by the various enzymes was found to vary from two to more than 20. The mixture of fragments produced by each restriction enzyme was tested for oncogenicity by s.c. injection into newborn hamsters within 24 hr of birth; 5 μg DNA was administered to each animal. That cleaved by *Eco*RI or *Hpa*I retained the ability to initiate tumors, whereas all the other preparations failed under the conditions tested; gel electrophoresis data preclude tumor induction *via* intact DNA. When tested separately, only the heavy fragments produced by *Eco*RI were oncogenic. In measuring tumor induction *in vivo* rather than transformation *in vitro*, no tumor induction was found with either intact Ad5 DNA or *Eco*RI fragments. In contrast to reports of transforming activity found in fragments of 10^6 dalton, no tumor-inducing ability was found with SA7 fragments smaller than 5×10^6 dalton, using either physically sheared material or restriction enzyme fragments. The results demonstrate that the information required for tumor induction is probably unique, and occurs only in the heavy half of the SA7 genome, expected near the central terminus.

0205 IDENTIFICATION OF EARLY ADENOVIRUS TYPE 2 RNA SPECIES TRANSCRIBED FROM THE LEFT-HAND END OF THE GENOME. (Eng.) Craig, E. A. (Wash. Univ. Sch. Medicine, St. Louis, Mo. 63110); McGrogan, M.; Mulder, C.; Raskas, H. J. *J. Virol.* 16(4):905-912; 1975.

Unique fragments of adenovirus type 2 DNA generated by cleavage with endonuclease R·*Eco* RI or endonuclease R·*Hsu* I (*Hin* dIII) were used to map cytoplasmic viral RNAs transcribed early in productive infection. Radioactive early viral RNA was first fractionated by polyacrylamide gel electrophoresis. Eluted viral RNAs were then tested for hybrid formation with DNA fragments. The *Eco* RI DNA fragment (*Eco* RI-A), which contains the left-hand 58% of the genome, hybridized 13S and 11S RNAs. More detailed mapping of these RNAs was achieved by hybridization to the seven *Hsu* I fragments of *Eco* RI-A. The early RNA annealed only to *Hsu* I-G and C, two fragments which comprise the extreme left-hand 17% of the genome. Viral RNA migrating as 13S and 11S annealed to *Hsu* I-G, and 13S RNA annealed to *Hsu* I-C. A 13S RNA is transcribed from *Eco* RI-A late in infection (18 hr). Hybridization-inhibition studies with *Eco* RI-A DNA, early cytoplasmic RNA, and ^{3}H-labeled 13S late RNA demonstrated that this RNA synthesized at late times is an early RNA species which continues to be synthesized in large amounts at 18 hr. This 13S RNA synthesized at 18 hr hybridized to *Hsu* I-C but not to

Hsu I-G DNA. These results establish that the 13S RNAs transcribed from *Hsu* I-G and C at early times must be different species.

0206 TERMINATION SITES FOR ADENOVIRUS TYPE 2 DNA REPLICATION. (Eng.) Tolun, A. (Wallenberg Lab., Uppsala Univ., Uppsala, Sweden); Pettersson, U. *J. Virol.* 16(4):759-766; 1975.

The termination points for adenovirus type 2 DNA replication were investigated. Cells were pulse-labeled 14 hr after infection for 5, 10, and 60 min. The total intracellular DNA was extracted, and the DNA was fractionated by chromatography on benzoyl-naphthoyl-DEAE-cellulose columns. Fractions eluted with 2% caffeine contained predominately DNA of a higher buoyant density than mature viral DNA. When DNA was cleaved with restriction endonuclease EndoR.*Eco*RI, a clear increase in the $^{3}H/^{32}P$ ratio was observed toward the right hand end of the genome, suggesting that one termination for replication is located at the right hand end of the Ad2 DNA. An increasing $^{3}H/^{32}P$ ratio was also observed toward the left hand end of the Ad2 DNA when the DNA was cleaved with EndoR.*Hpa*I, suggesting an additional left hand end terminus. Single strands of DNA generated in the presence of hydroxyurea also hybridized with both strands of viral DNA, indicating that both strands are exposed during replication. Thus, termination sites for replication of Ad2 DNA were detected at both ends of the genome; the single-stranded branches exposed during replication were characterized.

0207 DISTRIBUTION OF MURINE TYPE B AND TYPE C VIRAL NUCLEIC ACID SEQUENCES IN TEMPLATE ACTIVE AND TEMPLATE INACTIVE CHROMATIN. *(Eng.)* Howk, R. S. (Meloy Lab., Rockville, Md.); Anisowicz, A.; Silverman, A. Y.; Parks, W. P.; Scolnick, E. M. *Cell* 4(4):321-327; 1975.

The distribution of murine type B and type C viral nucleic acid sequences was studied in chromatin fractions which were active or inactive as *in vitro* templates for mammalian or *E. coli* RNA polymerase. The type B sequences were taken as an index for inactive DNA and the type C sequences were taken as a marker of DNA that is actively transcribed in the cell. Chromatin from NIH 3T3 mouse cells infected with the Moloney leukemia virus was fractionated by shearing and glycerol gradient sedimentation or by sonication and ectham cellulose chromatography. The two purification procedures yielded essentially identical results, the recovery of both the total DNA content of the chromatin and the total template activity of the chromatin being between 85 and 95% in each case. DNA-DNA reassociation kinetic analysis was performed using the DNA extracted from the template active and inactive chromatin and cDNA products obtained from the mouse mammary tumor type B virus or the Moloney strain type C virus; a cDNA to mouse globin mRNA was also used as a probe. The results indicated that the distribution of type B and type C sequences in the various fractions of the template active and template inactive chromatin were the same. These fractions also contained identical amounts of sequences homologous to the mouse globin cDNA. These studies demonstrate the usefulness of endogenous proviral genomes as probes for the structure of chromatin.

0208 RNA-DETECTED DNA POLYMERASE FROM HUMAN LEUKEMIC BLOOD CELLS AND FROM PRIMATE TYPE-C VIRUS-PRODUCING CELLS: HIGH- AND LOW-MOLECULAR-WEIGHT FORMS WITH VARIANT BIOCHEMICAL AND IMMUNOLOGICAL PROPERTIES. *(Eng.)* Mondal, H. (Natl. Cancer Inst., Bethesda, Md.); Gallagher, R. E.; Gallo, R. C. *Proc. Natl. Acad. Sci. USA* 72(3):1194-1198; 1975.

The RNA-directed DNA polymerase from gibbon ape leukemia virus, a gibbon ape virus-producing lymphosarcoma, and the blood leukocytes of a patient with acute myelogenous leukemia (AML) were subjected to velocity glycerol gradient analysis, and the primer-template responses and immunological properties of the glycerol gradient-purified enzymes were determined. The reverse transcriptase prepared from the AML leukocytes contained a low-molecular-weight (LMW) form (about 70,000 daltons) and a high-molecular weight (HMW) form (130,000-140,000 daltons). The enzyme from the extracellular gibbon ape leukemia virus contained only the LMW form, but the enzyme from the gibbon ape virus-producing lymphosarcoma cells contained some of the HMW form. The HMW form of the ape lymphosarcoma and human leukemia enzyme was converted completely to the LMW form by treatment with 0.5 M KCl and 0.5% Triton X-100, and could be reconverted to the LMW form by reduction of the KCl and Triton X-100 concentrations. The LMW form of the human and gibbon ape cellular enzymes utilized synthetic primer-templates in a fashion similar to that of viral enzyme, and this form was strongly inihibited by IgG antisera to reverse transcriptase from woolly monkey type-C virus. The HMW form of both enzymes utilized synthetic primer-templates less efficiently than the LMW form and was resistant to inhibition by the antiploymerase IgG of simian type-C virus. The HMW form of the cellular reverse transcriptases transcribed viral 70S RNA in the absence of synthetic primer more efficiently than did the extracellular viral form. The data suggest that the HMW form is due in part to aggregation of the HMW form and in part to a cellular factor(s) which may affect both the form and function of intracellular reverse transcriptase.

0209 DEFINITIVE EVIDENCE THAT THE MURINE C-TYPE VIRUS INDUCING LOCUS *AKV-1* VIRAL IS GENETIC MATERIAL. *(Eng.)* Chattopadhyay, S. K. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.); Rowe, W. P.; Teich, N. M.; Lowy, D. R. *Proc. Natl. Acad. Sci. USA* 72(3):906-910; 1975.

Inheritance of the *Akv-1* locus (one of the two murine leukemia virus [MuLV]-inducing loci of the AKR mouse strain) and the AKR-specific viral DNA sequences (DNA sequences homologous to a portion of MuLV RNA) was studied in AKR/J (*Akv-1*$^{+}$, *Gpi-1*^{b}, *c*), NIH Swiss

(*Akv-1*$^-$, *Gpi-1*^{b}, *c*), and C57Br/J (*Akv-1*$^-$, *Gpi-1*^{a}, *c*$^+$) mice, and hybrid crosses between these strains. Cellular DNA from 15 to 18-day embryos was hybridized with a viral [^{3}H] DNA probe and the segregation of viral sequences with virus inducibility was studied. When cellular DNA was hybridized with a Gross-AKR type MuLV probe, the virus-yielding and non-virus-yielding mouse strains differed with regard to: the proportion of probe sequences hybridized to the cell DNA; the number of classes of probe sequences represented in the cell DNA; and the thermal denaturation characteristics of the cell-probe hybrid molecules. Three-point genetic crosses involving *Akv-1*, *Gpi-1*, and *c* on individual mouse embryos indicated that a portion of the cell DNA hybridizable with the viral probe sequences segregated with *Akv-1*; this finding strongly suggested that the *Akv-1* locus contains viral sequences. Quantitation of the viral sequences in *Akv-1* congenic mice showed that the AKR DNA contains three to four copies of the AKR-specific viral sequences, while the NIH DNA contains only one class of viral sequences; in the case of the AKR DNA, the AKR-specific viral sequences were well matched to the viral [^{3}H] DNA, while those of the NIH DNA were poorly matched to the probe. The data suggest that the *Akv-1* virus-inducing locus may contain more than one copy of these sequences and that a larger number of copies may be the result, rather than the cause, of a high level of virus inducibility.

0210 EFFECT OF CORDYCEPIN ON THE REPLICATION OF TYPE-C RNA TUMOR VIRUSES. *(Eng.)* Richardson, L. S. (Litton Bionetics, Inc., Bethesda, Md.); Ting, R. C.; Gallo, R. C.; Wu, A. M. *Int. J. Cancer* 15(3):451-456; 1975.

The effect of cordycepin (3'-deoxyadenosine), which preferentially inhibits the iododeoxyuridine (IdU)-induced production of leukovirus from murine fibroblasts, on the replication of type C RNA tumor viruses was studied. IdU (40 μg/ml) was added to freshly plated or exponentially growing Kirsten murine sarcoma virus-transformed, nonproducer BALB/3T3 cells (K-BALB/3T3); the cultures were subsequently exposed to cordycepin (5-50 μg/ml). The effect of cordycepin on the transformation of normal rat kidney (NRK) cells by Kirsten murine sarcoma leukemia virus and Rauscher leukemia virus pseudotype (M-MSV[RLV]) was determined. Measurements of reverse transcriptase activity and focus-forming units (FFU) of virus were used to quantitate virus production, and the infectious center assay and measurements of antigen induction in the cells were used to determine the number of virus-producing cells. Cordycepin reduced the number of virus-producing cells in the IdU-induced cultures. In the M-MSV(RLV)-infected NRK cultures, 10 μg/ml of cordycepin inhibited viral production by 7 to 35 fold, but it reduced the number of FFU/plate from 240 to 141. Treatment of NRK cells with 5 μg/ml of cordycepin before infection with murine sarcoma-leukemia virus did not affect focus formation and reduced virus production by only two-fold, while exposure of the infected cells to cordycepin during the first 24 hr following infection caused a 24% decrease in the number of foci and a seven-fold decrease in total virus production; cordycepin treatment 24 to 48 hr after infection was less inhibitory. The mild cytotoxicity caused by low concentrations of cordycepin was probably not the main cause of reduced virus production in the treated cultures. The data indicate that cordycepin preferentially inhibits virus production, possibly by a mechanism acting after the formation of the provirus.

0211 HOST RANGE STUDIES OF FLOPC-1 MURINE MYELOMA C PARTICLES. (Eng.) Krueger, R. G. (Christ Hosp. Inst. Medical Res., 2141 Auburn Ave., Cincinnati, Ohio 45219). *J. Virol.* 16(5):1137-1145; 1975.

The host range of the C particle produced by FLOPC-1 myeloma cells, FLOPC-1 murine myeloma-associated virus (FL-MuMAV), was assessed in terms of its ability to productively infect and/or induce new viral antigens in a variety of different cell lines. Production of C particle-like structures by cells exposed to FL-MuMAV was determined by incorporation of [^{3}H]uridine into particles with a density of 1.16 g/ml and/or measurement of RNA-dependent DNA polymerase activity in concentrated culture medium. FL-MuMAV was capable of infecting NIH/3T3, normal rat kidney (NRK) cells, BALB/c 3T3, and the A_{31} clone of BALB/3T3 cells but not rabbit corneal (SIRC), implying that it is an N, B-tropic murine virus. Further, neoantigens, reactive with anti-FL-MuMAV serum, were detected on infected cells. Production of the MuMAV-like particle and MuMAV-associated cell antigens in infected NIH/3T3 and NRK cells persisted for three subcultures. The particles produced by infected NIH/3T3 or NRK cells were antigenically and physico-chemically similar to FL-MuMAV and not K-MuLV. The MuMAV-like particles produced by infected NIH/3T3 were capable of limited replication in NIH/3T3 and BALB/3T3 cells, whereas NRK-MuMAV replicated for a limited period in NIH/3T3, NRK, and SIRC cells; i.e., they had a different host range than FL-MuMAV. The particles produced by infected BALB/3T3 and A_{31} cells had the same host range as FL-MuMAV. In certain situations, isotopically labeled particles with a density of 1.16 g/ml were produced which appeared to lack RNA-dependent DNA polymerase. It is concluded that FL-MuMAV will infect NIH/3T3 for a limited period, that it probably infects BALB/3T3 cells, and that it does not infect SIRC cells. The particles produced possess antigenicity and RNA-directed DNA polymerase activity.

0212 EB VIRUS-ASSOCIATED NUCLEAR ANTIGEN PRODUCTION AND CELL PROLIFERATION IN ADULT PERIPHERAL BLOOD LEUKOCYTES INOCULATED WITH THE QIMR-WIL STRAIN OF EB VIRUS. (Eng.) Moss, D. J. (Queensland Inst. Med. Res., Herston, Australia); Pope, J. H. *Int. J. Cancer* 15(3):503-511; 1975.

A nuclear antigen, apparently the Epstein-Barr virus (EBV)-associated nuclear antigen (EBNA), was detected by anticomplement immunofluorescence (ACIF) tests in adult human peripheral blood WBC infected with the QIMR-WIL strain of EBV. Mononuclear prepara-

tions of WBC were made by a density gradient method. WBC were centrifuged and virus suspension (at dilution and multiplicity of infection) was added. A series of cultures were examined daily for four days following infection of EBV at a multiplicity of 0.005 TD_{50}/cell and plated out at 2.5 x 10^5/ml. EBNA was not detectable at 24 hr but appeared in about 11% of the cells by three days, and by five days up to 64% of the cells were positive. Proliferation of EBNA-positive cells at this stage was confirmed by autoradiography. There was a good correlation between the concentration of virus and the number of EBNA-positive cells in the first 5-7 days. The subsequent course of events was influenced by the initial cell concentration and time of subculture. EBNA production was delayed in cells infected with higher dilutions of virus but subsequently appeared in a high proportion of cells. Indirect immunofluorescence failed to detect viral capsid antigen or early antigen by ten days. The results show that EBV infection was abortive and that the critical events of viral transformation occurred within the first few days.

0213 LYMPHOBLASTOID TRANSFORMATION AND KINETICS OF APPEARANCE OF VIRAL NUCLEAR ANTIGEN (EBNA) IN CORD-BLOOD LYMPHOCYTES INFECTED BY EPSTEIN-BARR VIRUS. *(Eng.)* Yata, J. (Int. Agency Res. Cancer, Lyons, France); Desgranges, C.; Nakagawa, T.; Favre, M. C.; de-The, G. *Int. J. Cancer* 15(3):377-384; 1975.

The early events following Epstein-Barr virus (EBV) infection of total and separated B and T human cord-blood lymphocyte populations were studied. Undiluted cell-free EBV B95.8 culture supernatant or concentrated B95.8 virus was added to 10^6 lymphocytes before and after separation into B- and T-cell populations. The cultures were examined microscopically and were studied for the uptake of [^{3}H]thymidine, spontaneous rosette formation with sheep erythrocytes (E), and the formation of erythrocyte antibody complement complexes (EAC). The rosette-forming cells (RFC) were separated from the non-RFC and examined for surface immunoglobulin determinants and EBV nuclear antigen (EBNA). Lymphoblastoid cells appeared in the cell cultures about 2 days after infection and reached the 50% level by day 4; the proportion of lymphoblastoid cells was higher after infection with the cell-free supernatant than after infection with the concentrated virus. DNA stimulation was also greater after infection with the cell-free supernatant. The lymphoblastoid cells of the transformed cultures of the total population did not form E rosettes but were EAC-rosette-positive. When the lymphocytes were separated into RFC (T-cells) and non-RFC (B-cells + monocytes) populations prior to infection, lymphoblastoid cells formed almost exclusively in the non-PFC population. The total cultures contained 80% EBNA-positive cells 7 days after infection, the RFC population contained no EBNA-positive cells, and the non-RFC population contained primarily EBNA-positive cells 3 days after infection. UV light decreased the appearance of EBNA-positive cells in the total cell population. The data indicate that B-cells are susceptible to infection and transformation by EBV and that T-cells appear to be insusceptible to such infection and transformation.

0214 DEMONSTRATION OF A CELLULAR INHIBITOR OF EPSTEIN-BARR AND CYTOMEGALOVIRUS SYNTHESIS. (Eng.) Glaser, R. (Milton S. Hershey Med. Center, Pennsylvania State Univ., Hershey); Zimmerman, J.; St. Jeor, S.; Rapp, F. *Virology* 64(1):289-292; 1975.

A particle free extract prepared from human D98 cells was tested for inhibitory activity against the Epstein-Barr virus (EBV) and cytomegalovirus (CMV). The presence of a "host cell" mechanism controlling the expression of virus genetic material was investigated using an extract of D98 cells. The effect on the replication of EBV was determined using EBV "nonproducer" lymphoblastoid F-265 cells and the indirect immunofluorescent technique using EBV-specific antigens. When EBV-infected F-265 cells were exposed to D98 extract alone, there was a 20% and a 40% reduction in fluorescing cells; however, the simultaneous addition of poly-L-ornithine (Poly-O) resulted in a reduction of the number of positive cells by over 90%. The specificity of the inhibitor activity was tested using a second herpesvirus (cytomegalovirus). CMV-infected human embryo lung cell monolayers treated with D98 extract alone revealed a 24 hr delay in appearance of extracellular virus and a 100-fold reduction; cells treated with D98 and Poly-O also experienced a 24 hr delay, plus a 1,000-fold decrease in recovery of infectious virus. A particle free extract of D98 cells inhibited the replication of EBV and CMV. The results demonstrated that simple exposure of F-265 cells to 1,500 µg of protein extract is insufficient in preventing synthesis of EBV and CMV specific markers. The data suggest that D98 cells, which do not contain EBV genomes, inhibit the expression of EBV after acquisition of the genome, and inhibit the lytic replication of both EBV and CMV. The data also suggest that cellular-specified and/or virus-specified inhibitory factors may exist.

0215 ASSAY FOR EPSTEIN-BARR VIRUS BASED ON STIMULATION OF DNA SYNTHESIS IN MIXED LEUKOCYTES FROM HUMAN UMBILICAL CORD BLOOD. *(Eng.)* Robinson, J. (Yale Univ. Sch. Med., New Haven, Conn.); Miller, G. *J. Virol.* 15(5):1065-1072; 1975.

Relationships between the efficiency of DNA stimulation in cultured human umbilical cord WBC and the multiplicity of added Epstein-Barr virus (EBV) were investigated. Using EBV prepared from marmoset lymphoblastoid cell line, [^{3}H]thymidine ([^{3}H]Tdr) incorporation into acid-insoluble material was measured in infected and uninfected cultures. Following the kinetics of DNA synthesis in WBC cultures inoculated with serial dilutions of a virus stock, a direct relationship between the amount of added virus and the time before stimulation of [^{3}H]Tdr incorporation was evident. [^{3}H]Tdr uptake increased logarithmically to a maximum rate, whereas the cell count was not a sensitive indicator of transformation. Studies on small variations in the multiplicity of infection showed a linear relationship between the log of the amount of virus inoculated and

the log of the $[^3H]$Tdr incorporation measured. The capacity of diluted and undiluted EBV to stimulate $[^3H]$Tdr uptake was neutralized completely by EBV antibody; likewise, progressively larger doses of UV light applied to EBV stocks resulted in decreased DNA synthesis stimulation. Studies of the serum requirement revealed greatest stimulation in medium containing 20% fetal calf serum (FCS), with decreased stimulation in 10% and 5% FCS, and none in 2% FCS. Approximately 1% of the cell population is transformed at a multiplicity of 0.05 transforming units/cell. The speed, objectiveness, sensitivity, and reproducibility of the assay is noted, and the high efficiency of cell transformation by EBV is emphasized.

0216 ERYTHROPOIETIN-INDEPENDENT ERYTHROID COLONY FORMATION *IN VITRO* BY HEMOPOIETIC CELLS OF MICE INFECTED WITH FRIEND VIRUS. (Eng.) Liao, S.-K. (Fac. Med., Univ. Toronto, Canada); Axelrad, A. A. *Int. J. Cancer* 15(3):467-482; 1975.

The production of erythroid colonies in plasma culture by bone marrow and spleen cells taken from C3Hf/Bi mice previously infected with 5,000 focus-forming units of a polycythemic strain of Friend virus (FV) was investigated. The cultures were incubated for two days and harvested, and colonies of eight or more nucleated benzidine-staining cells were scored. Inclusion of 3 to 18 mU/0.1 ml of erythropoietin (Epo) in the medium was unnecessary for erythroid colonies by hemopoietic cells from normal animals. Development of erythroid colonies also proceeded unimpeded when cells from FV-infected animals were cultivated in medium pretreated with rabbit anti-serum that was shown to inactivate Epo. Thus, the hemopoietic tissues of FV-infected mice contained erythroid colony-forming units (CFU-Es) which appeared to be Epo-independent. When spleen cells from FV-infected mice were exposed to antiserum directed against syngeneic FV-infected spleen cells and complement, and then cultured with or without Epo, the number of erythroid colonies that developed was drastically reduced. This indicated that the CFU-Es in these animals carried FV-induced antigens, and must have been infected with virus. Electron microscopy of erythroid colonies produced by cells from FV-infected mice revealed the presence of budding and abundant free type-C virus particles. The efficiency of erythroid colony formation *in vitro* either with or without Epo by hemopoietic cells from FV-infected mice was substantially increased over that of cells from normal mice. The increase in number of CFU-Es in these animals was due mainly to an increase in the number of Epo independent CFU-Es. Epo-independent CFU-Es were first detected in bone marrow and spleen as early as three days after FV infection; thereafter their numbers progressively increased for at least nine days. Hypertransfusion with RBC prior to FV infection reduced, while bleeding greatly increased, the efficiency of erythroid colony formation without Epo by cells from the spleens of the infected mice. The phenomenon of erythroid colony formation in plasma cultures lacking Epo provides a sensitive and reliable means of detecting Epo-independent CFU-Es, which appear to play a fundamental part in pathogenesis of the disease resulting from infection with the polycythemic strain of FV.

0217 DIFFERENTIAL FEULGEN-DEOXYRIBONUCLEIC ACID HYDROLYSIS PATTERNS OF HERPES SIMPLEX VIRUS TYPE 1 AND TYPE 2 INFECTED CELLS. (Eng.) Trusal, L. R. (Dep. Biol., Pennsylvania State Univ., University Park); Anthony, A.; Docherty, J. J. *J. Histochem. Cytochem.* 23(4):283-288; 1975.

The Feulgen (F)-DNA hydrolysis rates of Herpes Simplex Virus (HSV)-1 and HSV-2-infected and uninfected human embryonic lung cells (HEL) grown in tissue culture were analyzed. HEL cells were infected with HSV-1 or HSV-2 at a multiplicity of infection of 20. The cells were incubated, rinsed and processed through a graded series of alcohols prior to 5 N HCl hydrolysis at room temperature. Cells were stained in Schiff's reagent and processed according to the Feulgen procedure. Microscopic examination of Feulgen-stained nuclei revealed a visibly discernible difference in the intensity and nuclear distribution of stained material in infected cells compared to uninfected controls. Infected nuclei were more densely stained, smaller and devoid of nucleoli. HSV-2-infected cells exhibited similar but more pronounced cytopathic alteration. The average percentage increase in F-DNA content of HSV-1 and HSV-2-infected cells over the control level was approximately 40 and 70%, respectively, over a 10-180 min hydrolysis interval. The average F-DNA level of type 2-infected cells was generally higher than that of type 1. Feulgen hydrolysis curves were analyzed and showed that there was a faster initial release of aldehydes in viral infected cells than in uninfected cells. The results support the conclusion that under standardized temperature and pH conditions, differences in initial slopes of Feulgen hydrolysis curves reflect differences in chromatin F-DNA levels. HSV-2 infected cells contain more F-DNA than HSV-1-infected or control cells. HSV-2 infected cells also exhibit the steepest initial hydrolysis curve slope.

0218 SYMMETRICAL TRANSCRIPTION OF HERPES SIMPLEX VIRUS DNA IN INFECTED BSC-1 CELLS. *(Eng.)* Ben Zeev, A. (Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel); Becker, Y. *Nature* 254 (5502):719-722; 1975.

The mode of transcription and the DNA strand selection for the transcription of herpes simplex virus (HSV) DNA were studied. BSC-1 green monkey kidney cell monolayer cultures were infected with the HF strain of HSV type 1 and 12 to 13 hr later were pulse labeled with ^{3}H-uridine. The total labeled RNA was extracted and the polyadenylic acid [poly (A)]-containing RNA molecules were isolated by cellulose column chromatography. About 12% of the total RNA was bound to the column and about 27% of the labeled RNA molecules synthesized in the nuclei of the infected cells were complementary and were able to form double-stranded RNA (dsRNA) molecules which were resistant to RNase treatment. Heating (100 C for 5 min) resulted in the denaturation of the dsRNA molecules and rendered them sensitive

to RNase treatment. The labeled dsRNA molecules banded at 1.622 g/ml in Cs_2SO_4, whereas the heat-denatured molecules banded at a density of 1.67 g/ml. The labeled RNA sedimented to the position of 4S and 10-16S in sucrose gradients, but the denatured molecules sedimented with a peak at about 18S. The main mass sedimented at 8-10S. The dsRNA hybridized to HSV DNA and not to BSC-1 DNA; however, only about 22.5% of the input RNA hybridized to the HSV DNA. The data indicate that the primary transcription products in the nuclei of HSV-infected BSC-1 cells are transcribed from the two strands of the viral DNA in a symmetrical fashion.

0219 MALIGNANT TRANSFORMATION OF HAMSTER CELLS FOLLOWING INFECTION WITH A BOVINE HERPESVIRUS (INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS). (Eng.) Michalski, F. (Yale Univ. Sch. Medicine, New Haven, Conn. 06510); Hsiung, G. D. *Proc. Soc. Exp. Biol. Med.* 148(3):891-896; 1975.

Experiments were conducted to determine whether or not infectious bovine rhinotracheitis (IBR) virus is capable of transforming cells *in vitro*. All virus stocks were prepared in bovine embryonic kidney (BEK) cell suspension. In 13 experiments using two oral strains of IBR virus and 30 experiments using two genital strains, foci of transformed cells were obtained in four separate incidences (three times in cells infected with genital isolates of IBR and once in cells infected with the oral strain). Morphologically, the transformed cells consisted primarily of fibroblast-like cells. There were abundant mitotic figures, many cells with multiple nucleoli, and clumped basophilic chromatin. Similar foci of morphologically transformed cells were noted in two experiments using a UV-irradiated inactivated genital strain IBR virus. Random-bred golden Syrian or white NIH inbred hamsters were inoculated sc with 10^5-10^7 transformed cells or 10^3-10^7 tumor cells and observed for tumor induction. Small solid tumors near the site of inoculation were noted in hamsters of both strains. Metastases to the lungs were found in 15% of the 73 tumors examined. Cells derived from the tumors propagated rapidly *in vitro* and showed a morphological resemblance to the transformed cells. Thus, the transformed cells were highly oncogenic. No infectious virus was found in either the transformed cells or in the tumor cells folowing cocultivation with BEK cells. IBR virus antigens or antibodies were detected by direct and indirect immunofluorescent techniques. Viral specific antigen was detected in about 5% of the transformed cells and in about 10% of primary tumor cells in culture. Viral specific antibody was detected in the serum of tumor-bearing hamsters, but no neutralizing antibodies were found.

0220 POLYAMINE METABOLISM IN CELLS INFECTED WITH HERPES SIMPLEX VIRUS. (Eng.) McCormick, F. P. (Dep. Biochem Univ. Cambridge, England); Newton, A. A. *J. Gen. Virol.* 27(1):25-33; 1975.

The synthesis of spermidine and spermine from putrescine in L cells grown in suspension cultures (LS cells) infected with Herpes Simplex Virus (HSV) was investigated in order to evaluate the significance of this pathway in the process of HSV replication. LS cells were harvested by centrifugation and HSV-1 in L cell medium (5 x 10^6 plaque-forming units/ml) was added to the cell pellet. The cells were labeled with [^{3}H]-putrescine (1 μCi/ml). The conversion of the added [^{3}H]-putrescine to spermidine was completely inhibited within five hr of infection of LS cells with HSV-1. Cells were labeled for 18 hr to allow a significant amount of radioactivity to accumulate in intracellular spermidine; five hr after infection of these cells the conversion of spermidine to spermine was inhibited. The conversion of putrescine to spermidine and of spermidine to spermine followed the same pathway as in other animal cells. When cycloheximide (20 μg/ml) or emetine was added to rapidly growing LS cells at the same time as [^{3}H]-labeled putrescine, the conversion of putrescine to spermidine and of spermidine to spermine was completely inhibited within five hr of adding the inhibitor. Putrescine, spermidine and spermine were lost for the cells about seven hr after infection. Methylglyoxal bis-(amidinohydrazone) (4 μM), a specific inhibitor of S-adenosyl methionine decarboxylase, inhibited spermidine and spermine synthesis when added to rapidly growing LS cells. The results suggest that uptake of putrescine is subject to a control process that is of general importance in cell metabolism, and that the alteration of this control by HSV-1 may be a significant feature in the transition to virus-directed metabolism in the infected cell.

0221 THE EFFECT OF RAUSCHER AND MOLONEY LEUKAEMIA VIRUS ON AMYLOID DEVELOPMENT IN CASEIN-TREATED CBA MICE. (Eng.) Ebbesen, P. (Inst. Med. Microbiol., Univ. Copenhagen, Denmark); Leuchars, E.; Doenhoff, M.; Nielsen, G.; Bergh, M.; Hesse, J. *Acta Pathol. Microbiol. Scand.* [*A*] 83(1):150-154; 1975.

The effect of Rauscher and Moloney leukemia virus on amyloid development in casein treated CBA mice was investigated. The mice were divided into four groups with different T-cell pools: unoperated control mice; mice thymectomized at eight weeks; adult thymectomized animals given 850 rads total body irradiation and 5 x 10^6 syngeneic bone marrow cells (T-cell deprived mice) and nonthymectomized irradiated mice given 5 x 10^6 syngeneic bone marrow cells (T-reconstituted mice). Repeated s.c. injections of a 5% solution of casein was administered eight weeks after irradiation and bone marrow reconstitution. In one experiment 1.5 x 10^3 XC test units (XC-units) Rauscher virus and, in another experiment, 1.3 x 10^4 XC-units Moloney virus were given i.p. once, simultaneously with initiation of casein treatment. Amyloid, if present, was only found in the spleen; no amyloid was present in mice not given casein. There were no significant differences in the degree of amyloid in any of the four groups of animals in both experiments. Mice with leukemia infiltrates had neither more nor less amyloid than other mice in their group. No statistically significant difference in leukocyte counts in the various experimental groups was found. Caseinated irradiated

mice had 10^3 times more virus per gram of spleen than saline-treated irradiated mice. Bone marrow cell cultures derived from virus-infected caseinated mice differed less than 20% from cultures derived from caseinated, noninfected mice with regard to number of cells surviving after one week. Peroxidase staining showed the presence of polymorphonuclear granulocytes and monocytes in all cultures. The involvement of the bone marrow population in amyloidogenesis is as yet virtually unexplored; the importance of bone marrow reactions for viral interference with amyloidogenesis therefore cannot be elucidated from the present results.

0222 THE ENDOGENOUS REVERSE TRANSCRIPTASE ACTIVITY OF GIBBON APE LYMPHOMA VIRUS: CHARACTERIZATION OF THE DNA PRODUCT. (Eng.) Harewood, K. R. (John L. Smith Memorial for Cancer Res., Pfizer Inc., 199 Maywood Ave., Maywood, N. J. 07607); Chang, P.; Higdon, C.; Larson, D. *Biochim. Biophys. Acta* 407(1):14-23; 1975.

The DNA product of the endogenous reverse transcriptase reaction of Gibbon ape lymphoma virus (GALV) was analyzed and characterized. In simultaneous detection assays in which the type and/or concentration of divalent cation was varied, the best yield of rapidly-sedimenting DNA was obtained from reactions containing 1.5 mM Mn^{2+}. This yield was ten-fold better than the yield observed at the optimal Mg^{2+} concentration (5.0 mM). DNA synthesized at the optimal concentration of either of these cations consisted of large pieces varying in size from 4 to 12S. This DNA hybridized efficiently to homologous viral RNA (greater than 60% annealing) and protected at least two-thirds of GALV 70S [^{32}P]RNA from ribonuclease digestion. The hybrids formed with homologous viral RNA were stable as evidenced by their thermal elution patterns from hydroxylapatite columns. In contrast, DNA synthesized in reactions in which the concentration of Mn^{2+} or Mg^{2+} was greater than optimal was predominantly 4S or smaller in size and displayed a low level of hybridization (less than 10%) to homologous viral RNA.

0223 ESTABLISHMENT OF TWO CANINE SARCOMA CELL LINES: PRODUCTIVE INFECTION WITH FELINE LEUKEMIA VIRUS. (Eng.) Chapman, A. L. (Univ. Kansas Medical Center, Kansas City, Kans. 66103); Fischinger, P. J.; Tung, H.-N. *J. Natl. Cancer Inst.* 55(2):345-352; 1975.

Two sarcoma cell lines (11028, 11031), derived from the cervical lymph node of a 10-mo-old male dog and from a metastastic tumor in the liver of a 1-yr-old male dog, were established *in vitro* and have been transferred 213 and 306 times, respectively, since 1970. These cell lines had a chromosome pattern consistent with their canine origin. Both cultures were infected with feline leukemia virus (FeLV), which caused morphologic and karyotypic changes. The cells became rounded after infection with FeLV, and both cultures showed the presence of a single, large acrocentric chromosome considered to be a marker chromosome. All tumors were transplanted into newborn beagle pups, but only the 11028-FeLV formed metastatic tumors. No helper or focus-forming activity or virus particles were found in the uninfected cultures. Helper activity and virus were demonstrated in both sarcoma lines after infection with FeLV, though no focus-forming activity was noted. Helper activity of progeny virus could be assayed on either cat or dog embryo cells.

0224 BIOCHEMICAL PROPERTIES AND REPLICATION OF MURINE INTRACISTERNAL A PARTICLES DURING EARLY EMBRYOGENESIS. *(Eng.)* Yang, S. S. (Natl. Cancer Inst., Bethesda, Md.); Calarco, P. G.; Wivel, N. A. *Eur. J. Cancer* 11(3);131-138; 1975.

The appearance, fate, and biochemical characteristics of intracisternal A particles (IAP) in preimplantation mouse embryos were studied. Intracisternal A particles were isolated and purified from CF-1 oocytes and Swiss Webster, ICR, BALB/c, CF-1, New Zealand Black, and AKR preimplantation embryos. These particles and IAP isolated from the MOPC-104E mouse plasma cell tumor line were analyzed to determine their nucleic acid makeup, their DNA polymerase activities, and the kinetics and products of the endogenous reaction of their RNA-dependent DNA polymerase. IAP were seen in small numbers in the agranular endoplasmic reticulum of dictyate oocytes, but were rarely observed in oocytes after breakdown of the germinal vesicle had begun; the number of particles seemed to peak at the 4-8 cell stage. A particle formation was also observed in the first polar body during the late 2-cell stage and in the surviving second polar body during the blastocyst stage. IAP were readily obtained from the N-18 neuroblastoma cells. Purified IAP exhibited high molecular wt 60-70 S RNA and an endogenous RNA-dependent DNA polymerase which could be stimulated to much greater activity by the addition of exogenous templates. Requirements for maximal endogenous activity included the presence of dithiothreitol, Mn^{2+} or Mg^{2+}, Na^+ or K^+, and all four deoxyribonucleoside triphosphates. Of the synthetic templates studied, $dT^{12} \cdot rA^n$, poly dT·poly rA, and oligo $dG^{12} \cdot$poly rC stimulated the A particle DNA polymerase, while $dT^{12} \cdot dA^n$ and poly d(A-T) did not. The endogenous reaction of the IAP RNA-dependent DNA polymerase produced a DNA transcript of its intrinsic RNA with base complementarity. The results suggest that IAP are transmitted vertically and that the information for gene expression is present in the maternal ovum. Crystalloid material appearing at the time of the IAP appears to be related to the A particles.

0225 INDUCTION OF ERYTHROID DIFFERENTIATION IN MURINE VIRUS INFECTED ERYTHROLEUKEMIA CELLS BY HIGHLY POLAR COMPOUNDS. *(Eng.)* Tanaka, M. (Dep. Med., Columbia Univ., New York, N.Y.); Levy, J.; Terada, M.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. USA* 72(3):1003-1006; 1975.

The ability of various agents, related only indirectly in terms of structure and properties to dimethylsulfoxide (Me_2SO) and *N,N*-dimethylformamide, to induce the differentiation of murine-virus-infected erythro-

leukemia cells (MELC) to erythroid cells was studied. MELC strain 745 A was cultured in medium containing the various compounds in concentrations ranging from 15 mM or less to a concentration at which the compound inhibited the growth of MELC. Cell number, the proportion of viable cells, and the proportion of Hb-containing cells were determined. In addition to Me_2SO and *N,N*-dimethylformamide, 1-methyl-21-piperidone, *N-N*-dimethylacetamide, *N*-methylpyrrolidinone, *N*-methylacetamide, 2-pyrrolidinone, propionamide, pyridine-*N*-oxide, piperidone, *N*-methylformamide, acetamide, and triethylene glycol induced MELC to erythroid cell differentiation. 1-Methyl-2-piperidone showed the lowest concentration optimum (10 mM) and Me_2SO, acetamide, and triethylene glycol showed the highest concentration optimum (250-280 mM) for induction. For all compounds, the ratio of the lethal concentration to the optimal concentration for induction was similar (mean 1.8). There was no correlation between dipole moment and the concentration optimum for induction of these compounds. Urea (15-280 mM), acetone (10-100 mM), hexamethylphosphoric triamide (15-140 mM), ethylene carbonate (3-250 mM), and pyridine (3-250 mM) did not induce MELC to erythroid differentiation. These data in conjunction with previous findings suggest that the compounds which can induce MELC to differentiate may act by changing the conformation of DNA or DNA-protein complexes and initiating transcription of the gene(s) that regulates expression of the erythroid cell program.

0226 CORRELATION OF EARLY MURINE LEUKEMIA VIRUS TITER AND *H*-2 TYPE WITH SPONTANEOUS LEUKEMIA IN MICE OF THE BALB/c x AKR CROSS: A GENETIC ANALYSIS. (Eng.) Lilly, F. (Albert Einstein Coll. Med., Bronx, N.Y.); Duran-Reynals, M. L.; Rowe, W. P. *J. Exp. Med.* 141(4):882-889; 1975.

The relationship between the expression of endogenous murine leukemia virus (MuLV) and spontaneous leukemia in a segregating population of mice was investigated. All of the mice possessed two or more copies of the *Akv* genes, which had previously been shown to be a determinant of MuLV expression, but varied genotypically in respect to regulatory genes capable of interfering with the expression of the disease. MuLV testing and H-2 typing were carried out on 335 (BALB/c x AKR)F_1 x AKR backcross mice. XC cell plaque assay was conducted on 1 cm terminal tail segments of six week-old mice. The mice were then observed for the development of leukemia, until the youngest was 600 days old. When *in extremis* the mice were sacrificed and autopsied. Of the mice observed, 33.1% developed leukemia during the 19 to 21 month observation period. Of these, 55.5% of the deaths occurred among mice which had shown detectable levels of MuLV in the XC cell plaque assay; 8.7% of the virus-negative mice died of leukemia. A hemagglutination method was used to determine the H-2 type of the mice. Leukemia developed in 42.8% of the H-2^k/H-2^k homozygotes, but in only 23.7% of the H-2^k/H-2^d heterozygotes. While both early MuLV presence and H-2 type can be correlated with the expression of leukemia, it appears that H-2 type has little influence on the early expression of the virus. No statistically significant difference in occurrence could be attributed to sex, even though sex is significant in the AKR parent.

0227 INHIBITION OF RIBONUCLEIC ACID-DIRECTED DEOXYRIBONUCLEIC ACID POLYMERASE OF MURINE LEUKEMIA VIRUS BY POLYRIBONUCLEOTIDES AND THEIR 2'*O*-METHYLATED DERIVATIVES. (Eng.) Arya, S. K.; (Dept. Medical Viral Oncology, Roswell Park Memorial Inst., Buffalo, N.Y. 14203); Carter, W. A.; Alderfer, J. L.; Ts'o, P. O. P. *Mol. Pharmacol.* 11(4): 421-426; 1975.

The inhibitory effect of polyribonucleotides and their 2'-*O*-methylated derivatives on the kinetics of RNA-directed DNA polymerase of detergent-disrupted Moloney murine leukemia virus (MLV) was studied. MLV was obtained from infected JLS-V9 cells and purified by sucrose gradient centrifugation. Poly(inosinic acid) [poly(I)] and poly(2'-*O*-methylinosinic acid) [poly(Im)] strongly inhibited the polymerase activity, whereas poly(uridylic acid) [poly(U)] and and poly(2'-*O*-methyluridylic acid) [poly(Um)] showed a lesser degree of inhibition. Poly(cytidylic acid) and poly(2'-*O*-methylcytidylic acid) failed to show any significant inhibition. With poly(A):oligo(dT) as the template:primer at a concentration of 10:1 μM, 0.6 μM and 15 μM of poly(I) and poly(Im), respectively, were required to achieve 50% inhibition of polymerase activity. When the poly(A):oligo(dT) concentration was doubled, the poly(I) and poly(Im) concentrations required for 50% inhibition also doubled. With poly(C):oligo(dG) as a template: primer at a concentration of 5:0.5 μM, the concentration of poly(U) and poly(Um) required for 50% inhibition of polymerase activity was 60 μM and 90 μM, respectively. Reducing the reaction mixture resulted in a corresponding reduction in the poly(U) and poly(Um) concentration required for 50% inhibition. Thus, the degree of inhibition was linearly proportional to the polyribonucleotide concentration. However, at higher values of fractional inhibition, the degree of inhibition was not a linear function of the polyribonucleotide concentration, suggesting that the polyribonucleotides are not simple competitive inhibitors of the template:primer. This idea was further substantiated by evaluation of the inhibition kinetics by the Lineweaver-Burk and Dixon plots. The authors conclude that the potency of the inhibitor depends on the base composition of the polyribonucleotide, with polyinosinic acids being more potent inhibitors than polyuridylic acids.

0228 POLYPEPTIDES OF THE MOUSE MAMMARY TUMOR VIRUS. I. CHARACTERIZATION OF TWO GROUP-SPECIFIC ANTIGENS. *(Eng.)* Sarkar, N. H. (Inst. Med. Res., Camden, N.J.); Dion, A. S. *Virology* 64 (2):471-491; 1975.

The polypeptide composition of the mouse mammary tumor virus (MuMTV) was analyzed by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Intact or disrupted virions from RIII mouse milk were labeled with ^{125}I. Mammary tumor cells from BALB/cfC3H mice were grown in tissue culture. In some experiments these cells were labeled with ^{14}C-labeled L-amino acid mixture

and [^{3}H]glucosamine. Purified intracytoplasmic A particles from spontaneous lymphoid leukemias of DBA/2 mice were also analyzed. SDS-PAGE of MuMTV reproducibly resolved at least 11 polypeptides, the molecular weights of the major polypeptides being 55,000 (p55), 34,000 (p34), 28,000 (p28), 18,000 (p18), and 12,000 (p12). P55 and p34 were characterized as glycoproteins on the basis of staining with periodic acid-Schiff reagent and the incorporation of labeled glucosamine. The intracytoplasmic A particles contained five polypeptides of molecular weight 52,000 (Ap52), 43,000 (Ap43), 37,000 (Ap37), 29,000 (Ap29), and 15,000 (Ap15). Antisera against p55 were able to precipitate intact virus. Immunoelectron microscopy indicated that polypeptide p55 is a component of the viral surface. Antigen p55 was the most prominent viral protein and was common to viruses isolated from RIII, C3H, GR, and A mice. Polypeptide 28 was not a glycoprotein and antisera against purified p28 did not react to the viral surface. Immunodiffusion assays indicated that p55 and p28 were immunologically unique and that p28 shared a common antigen determinant with the intracytoplasmic A particles, probably with Ap29. P28 was a common antigen in the RIII, C3H, GR, and A viruses. The data indicate that p55 is a component of the viral surface and that it is a group-specific antigen (gs-1) of MuMTV. In contrast, p28 is most likely a major group-specific internal antigen (gs-2) of MuMTV.

0229 INHIBITION OF ONCORNAVIRAL DNA POLYMERASES BY 5-MERCAPTO POLYCYTIDYLIC ACID: MODE OF ACTION. (Eng.) Chandra, P. (Gustav-Embden-Zentrum der Biologischen Chemie, Abteilung für Molekularbiologie der J. W. Goethe-Universität, Frankfurt, West Germany); Ebener, U.; Götz, A. *FEBS Lett.* 53(1):10-14; 1975.

The mode of action of a partially thiolated polycytidylic acid (MPC) in the DNA-polymerase reaction of Friend Leukemia virions (FLV) was investigated. Spleens from FLV-infected AKR mice were used to isolate virions. The virion-associated DNA-polymerase activity was measured by [^{3}H] methyl-dTMP or [^{3}H]dGMP incorporation into DNA. Viral DNA polymerases required a primer molecule, with a free 3-OH end for copying of single-stranded regions of templates from the double-stranded product. The two synthetic template primers that appeared to be relatively specific on oncornaviral reverse transcriptases were oligo (dG) x poly (rC) and oligo (dT) x poly (rA). The incorporation of [^{3}H]dGMP into DNA by the viral enzyme was stimulated 3- to 9-fold in the presence of oligo (dG) x poly (rC). Under similar experimental conditions oligo (dG) x (MPC) failed to stimulate the [^{3}H]dGMP incorporation into DNA. The addition of 1-4 μg of oligo (dG) x poly (rC) in the reaction mixture lead to a slight increase of [^{3}H] dGMP incorporation. The effect of oligo (dG) x (MPC) on the template functions of unmodified duplex, oligo (dG) x poly (rC) was influenced at higher enzyme concentrations. Analysis of the DNA species synthesized by FLV-DNA polymerase was made by elution from hydroxylapatite column. In the presence of MPC there was an over-all inhibition of [^{3}H]dTMP incorporation, indicating that the formation of all three DNA species was blocked. The data indicate that the partially thiolated poly C is functioning as a dead template in the DNA polymerase system of FLV.

0230 CHROMATOGRAPHIC SEPARATION AND ANTIGENIC ANALYSIS OF PROTEINS OF THE ONCORNAVIRUSES. III. AVIAN VIRAL PROTEINS WITH GROUP-SPECIFIC ANTIGENICITY. *(Eng.)* Fletcher, P. (Sloan-Kettering Inst. Cancer Res., New York, N.Y.); Nowinski, R. C.; Tress, E.; Fleissner, E. *Virology* 64(2):358-366; 1975.

A comparitive amino acid analysis of group-specific proteins from two avian viral isolates is described. ^{35}S-methionine-labeled MC29 avian leukosis virus (AvLV) was obtained from myeloblasts infected with avian myeloblastosis viruses (AMV). Four of the seven prominent proteins resolved are nonglycoproteins (p27, p19, p15 and p12). By single amino acid labeling, p19 and p12 were found to be lysine- and arginine-rich, resp.; these proteins also had the highest overall content of hydrophilic amino acids. Proteins p27 and p15 showed a substantial content of branched-chain, hydrophobic amino acids and lower hydrophilicity. Peptides with identical mobilities using thin-layer electrophoresis were identifiable in the digests of p27, p19 and p15. The p12 was unrelated. Using gel filtration, tryptic peptides of AMV proteins showed that p27 was different from p19 and p15. In contrast, a certain number of p15 peptides could be matched with p19 peptides in terms of elution position and peak height. These results indicate that proteins of two AvLV isolates are similar, but do not have a close relationship in primary structure. Avian oncornaviruses appear to possess an additional polypeptide sequence (p19) compared with mammalian and reptilian oncornaviruses. The selective advantage of this extra protein is postulated to maintain the presence of group-reactive antigen recognition sites.

0231 ACQUISITION OF VIRAL DNA SEQUENCES IN TARGET ORGANS OF CHICKENS INFECTED WITH AVIAN MYELOBLASTOSIS VIRUS. (Eng.) Shoyab, M. (Univ. California Sch. Medicine, Los Angeles, Calif. 90024); Baluda, M. A. *J. Virol.* 16(4):783-789; 1975.

The distribution of oncornavirus DNA sequences in various tissues of normal chickens and of chickens with leukemia or kidney tumors induced by avian myeloblastosis virus (AMV) was analyzed by DNA-RNA hybridization using 35S AMV RNA as a probe. All the tissues from normal chickens which were tested contained the same average cellular concentration of endogenous oncornavirus DNA (1212 cpm hybridized per 100 μg cellular DNA). In contrast, different tissues from leukemic chickens and from chickens bearing kidney tumors contained different concentrations of AMV homologous DNA: in some tissues there was no increase whereas other tissues acquired additional AMV-specific DNA sequences. The increase (2- to 2.5-fold) was the greatest in tissues which can become neoplastic after infection, such as myeloblasts, RBC, and kidney cells. It was directly demonstrated that DNA from AMV-induced kidney tumor contains AMV sequences which are absent in DNA from normal cells.

A similar finding had been previously obtained with leukemic cells. ^{3}H-labeled 35S RNA from purified AMV was exhaustively hybridized with an excess of normal chicken DNA to remove all the viral RNA sequences which are complementary to DNA from uninfected cells. The ^{3}H-labeled RNA which failed to hybridize was isolated by hydroxylapatite column chromatography which separates DNA-RNA hybrids from single-stranded RNA. The residual RNA hybridized to chicken kidney tumor DNA but did not rehybridize with normal chicken DNA.

0232 GROUP-SPECIFIC ANTIGENIC DETERMINANTS OF THE LARGE ENVELOPE GLYCOPROTEIN OF AVIAN ONCORNAVIRUSES. (Eng.) Rohrschneider, L. (Institut fur Virologie, Fachbereich Human-medizin, 6300 Giessen, Germany); Bauer, H.; Bolognesi, D. P. *Virology* 67(1):234-241; 1975.

Various serological determinants in the major envelope glycoprotein (gp85) were characterized. Gp85 was isolated from avian myeloblastosis virus (AMV) obtained from leukemic chickens and from the Prague strain of Rous sarcoma virus of subgroup C (PR-RSV-C). Viruses used for the immunization of chickens and the neutralization tests were: myeloblastosis-associated virus of subgroup B and the Schmidt-Ruppin strain of RSV of subgroup D (SR-RSV-D). The glycoprotein was purified by both velocity and equilibrium sedimentation in sucrose density gradients. Immune precipitation demonstrated that antibodies recognized antigenic determinants in gp85 of virus from distinct subgroups; the precipitation of gp85 of PR-RSV-A virus by anti-SR-RSV-A antisera was a homologous reaction, whereas the precipitation of gp85 of PR-RSV-A by anti-MAV-B antisera was heterologous. Sites on envelope glycoproteins that were recognized by sera from infected chickens were distinct from those sites involved in virus neutralization and interference. Immunodiffusion with both the gp85 from AMV-B and from PR-RSV-C and their respective antisera confirmed the existence of a common antigenic determinant in gp85 molecules from viruses of two different subgroups. Radioimmunoassays of the serological reactivity of AMV and PR-RSV-C gp85 and competition assays both indicated that these glycoproteins contain prominent cross-reacting determinants. Thus, the existence of group-specific antigenic determinants contained in gp85 AMV was demonstrated by three different immunological techniques.

0233 ELECTRON MICROSCOPY OF VIRAL RNA: AVIAN TUMOR VIRUS RNA. (Eng.) Chi, Y. Y. (Upstate Med. Cent., State Univ. New York, Syracuse); Bassel, A. R. *Virology* 64(1):217-227; 1975.

The structure of native, partially denatured, and heated and quick-cooled RNA from avian myeloblastosis virus (AMV) and Schmidt-Ruppin Rous sarcoma virus (SR-RSV) was investigated by electron microscopy. BAI-strain A AMV-containing chicken plasma and Schmidt-Ruppin RSV of subgroup B, propagated on secondary cultures of C/O chick embryo fibroblasts, was purified and prepared for electron microscopy. In the sedimentation patterns of native RNAs of AMV and SR-RSV, material corresponding to 28 and 18 S ribosomal RNA was present in both gradients. When AMV or SR-RSV 60-70S RNA was prepared for electron microscopy by an aqueous nondenaturing method, uniform "folded" structures were seen. Heating the 60-70S RNA at 80 C for two min prior to preparation revealed smaller molecules still containing abundant secondary structure; the lack of uniform size or pattern indicated a heterogeneous population of molecules. Partially denatured AMV 60-70S RNA revealed some extended RNA structures; preparation using the formaldehyde-formamide method, causing dissociation and loss of secondary structure, resulted in extended RNAs and strands of differing lengths in both viruses. Preparation of the AMV 30-40S RNA by the formaldehyde-formamide procedure yielded extended strands of more uniform length. The large number of strands shorter than 2 μm may be due to "hidden" breaks, purification-induced fragmentation, or subunits smaller than 2.1 μm. The length distribution of strands of 60-70S SR-RSV RNA again revealed a majority of strands shorter than 1 μm, but with a different pattern of longer strands than the AMV RNA. In contrast to previous findings, large, uniformed-sized, folded molecules of 60-70S RNA of AMV or SR-RSV were found, with an RNA folded structure similar to that of other single-stranded cellular and viral RNAs. The results indicate that the 2.9×10^6 dalton subunit of AMV corresponds to the *b* subunit of nontransforming viruses, and the 3.5 $\times 10^6$ dalton subunit of SR-RSV corresponds to the *a* subunit of transforming viruses.

0234 CHICKEN LEUKOSIS VIRUS GENOME SEQUENCES IN DNA FROM NORMAL CHICK CELLS AND VIRUS-INDUCED BURSAL LYMPHOMAS. (Eng.) Neiman, P. E. (Univ. Washington Sch. Med., Seattle); Purchase, H. G.; Okazaki, W. *Cell* 4(4):311-319; 1975.

Genome sequences of two recent field isolates of avian leukosis viruses (ALV) in the DNA of normal and neoplastic white leghorn chicken cells were studied by DNA-RNA hybridization under conditions of DNA excess. ALV genome sequences in the DNA of virus-induced lymphomas were detected by hybridization with ALV-60-70S ^{125}I-labeled RNA. About 70% of the radioactive leukosis virus RNA in both instances entered ribonuclease-resistant hybrids at Cot levels above 4×10^4 (Co is the bulk DNA nucleotide concentration and t is the time in seconds). Labeled RNA from the endogeneous virus formed hybrids with DNA from normal chick embryos as extensively as it had with tumor DNA, but at a slightly slower rate reaching 70% at Cot values of about 7 to 8×10^4. Comparisons were made between 60-70S RNA from these viruses and that of a chicken endogenous type C virus (RAV-0) and of a series of "laboratory" leukosis and sarcoma viruses, by competitive hybridization analysis. Unlabeled viral RNA was added to the reaction mixtures to form a RNA excess. Theoretical curves for competition by homologous RNA were obtained by observing the decrement in extent of hybridization predicted by the computer program in increasing the RNA:DNA ratio in the reaction mixture. A minimum of 18% of the genome sequences of both ALV isolates detected in DNA from lymphomas they induced were not detected in normal chicken DNA. The vast majority of the fraction of RNA sequences from ALV which did form

hybrids with normal chick DNA appeared to be reacting with the endogenous provirus of RAV-0. The genomic representation of a variety of avian leukosis and sarcoma viruses in normal chicken cells could not be distinguished by these methods. In contrast, the portion of the ALV genome exogenous to the normal chicken genome showed significant divergence from that of two sarcoma viruses. The increased hybridization of ALV RNA with lymphoma DNA was used to detect the appearance of ALV specific sequences in the bursa of Fabricius following infection. It is concluded that the more extensive hybridization of ALV 60-70S RNA with DNA from virus induced lymphomas is caused by the insertion of new viral genes.

0235 MURINE ONCORNAVIRUS HIGH-MOLECULAR-WEIGHT RNA STRUCTURE: THERMAL STEPWISE DISSOCIATION OF 70S MURINE LEUKEMIA-SARCOMA VIRUS TO SUBUNITS AND LOW-MOLECULAR-WEIGHT ASSOCIATED RNAS. (Eng.) Emanoil-Ravicovitch, R. (Institut de Recherches sur les Leucémies et les Maladies du Sang, Hôpital Saint-Louis, 75010, Paris, France); Robert-Robin, J; Bazilier, M.; Boiron, M. *J. Virol.* 15(4):714-719; 1975.

The thermal dissociation into subunits, and the low-molecular weight-associated RNAs of the aggregate structure of the 70S RNA of a murine leukemia sarcoma [M-MSL(MLV)] viral complex was studied. The genome structure was investigated by following the conversion, at various temperatures, of the 70S RNA into smaller components *via* polyacrylamide gel electrophoresis. Using mouse sarcoma virus (Moloney strain) prepared from growth fluids of the chronically infected cell line 78A, thermal stepwise dissociation revealed intermediate RNA components, and complete dissociation of the 70S RNA molecule at 80 C. Two major components with electrophoretic mobilities corresponding to molecular weights of about 5×10^6 (I RNA) and 3.2×10^6 (II RNA) were then revealed; the light-associated RNA's released by total denaturation exhibited 8% of the native 70S RNA radioactivity. Exploring the associative nature of each light RNA released *via* heating at various temperatures, revealed that light RNAs associated with the M-MSL(MLV) genome are the 8S, 5.5S, 5S, and 4S RNAs; the 4S species appeared at rather low temperatures; the 5 and 5.5S RNAs appeared when the 70S RNA disappeared. More extensive characterization, using calculated T_m values of each RNA species released, revealed that not all of the light associated RNAs release is concomitant with the dissociation of the 70S RNA into subunits. The experiments demonstrated the aggregated structure of the mouse sarcoma leukemia viral complex. The stepwise release of light RNAs upon thermal treatment indicated that the nature of their association with the different RNA species of the genome is different for each light RNA. It was suggested that both I RNA and II RNA populations are simultaneously formed with a certain lag phase in the 70S RNA dissociation. It was concluded that the function of the majority of 70S-associated low molecular weight RNAs was to maintain the overall aggregate structure of the viral genome, the subunits of which are held together by uneven hydrogen bonds.

0236 CHROMATOGRAPHIC SEPARATION AND ANTIGENIC ANALYSIS OF PROTEINS OF THE ONCORNAVIRUSES. IV. BIOCHEMICAL TYPING OF MURINE VIRAL PROTEINS. *(Eng.)* Buchhagen, D. L. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Stutman, O.; Fleissner, E. *J. Virol.* 15(5):1148-1157; 1975.

Tryptic peptide maps were prepared for p10, p12, p15, and p30 derived from several murine leukemia viruses (MuLV). Using initial stocks of R-MuLV, M-MuLV, and G-MuLV, secondary cell cultures were infected with virus. These were radiolabeled *via* 16 hr exposure to radioactive precursors in the growth medium. The virus was isolated, purified, and subjected to polyacrylamide gel electrophoresis, trypsin digestion, and chromatographic peptide separation. The polypeptide profile of purified [^{3}H]-labeled L-amino acid-labeled R-MuLV and [^{14}C]-labeled L-amino acid labeled M-MuLV revealed that for the major polypeptides, a marked difference occurred only in the region of the major viral genome. However, the elution patterns of [^{3}H]-R-MuLV, [^{14}C]-G-MuLV, and [^{3}H]-M-MuLV indicated that the R-MuLV and M-MuLV polypeptides are of higher molecular weights than the corresponding G-MuLV peptide. The polypeptide p10, found associated with the viral RNA, appeared of identical size for all three. Tryptic analysis of p30 and resultant peptide profiles showed that the [^{3}H] R-MuLV and [^{3}H]-M-MuLV p30s were nearly identical to the [^{14}C]-G-MuLV p30 profile. However, the peptide maps of R-MuLV and G-MuLV were very dissimilar for their respective p15s; a lack of homogeny for the p12 proteins of the three viruses was also noted. A higher degree of homogeny was observed for the purified p10s. Co-chromatography of p30 peptides derived from two BALB/c viruses revealed extensive structural relatedness, yet suggested the p30 sequence may be subject to especially frequent type-specific variation. Thus, the biochemical approach revealed that the p30 and p10 proteins of Rauscher, Moloney, and Gross MuLV's are quite similar, that the p15 and p12 appear strain-specific, and that unique peptides also appear in each.

0237 PROLIFERATION KINETICS OF A VIRUS-INDUCED SARCOMA IN THE RAT KIDNEY. *(Eng.)* Zobl, H. (Dep. Pathol. Med. Sch., Hanover, West Germany); Lang, W.; Georgii, A. *Eur. J. Cancer* 11(3):159-167; 1975.

The kinetics of tumor growth were studied in Polyomavirus-induced autochthonous fibrosarcomas of the rat kidney. Newborn Wistar rats were inoculated s.c. with 0.3 ml of a virus suspension. The inoculated rats were studied at 30, 40, and 50 days of age to determine the percentage of labeled mitoses following a single i.p. dose of ^{3}H-thymidine (^{3}H-TdR), and to determine the tumor growth curve, cell cycle time and phase duration, proliferative fraction (PF), and rate of cell loss following 40-70 hr of continuous i.v. ^{3}H-TdR infusion. About 20 days after infection, richly vascularized, scarcely fiber-producing fibrosarcomas developed predominantly in the outer zone of the renal medulla. The tumor doubling time was 47 hr 30 days after virus inocu-

lation and 72 hr after 40 days. The mean cell cycle time was 21.1 hr by the tenth day, 26.3 hr by the twentieth day, and 29.8 hr by the thirtieth day of growth; the minimal G_2 length was shorter than 1 hr. The PF ranged between 39% and 50% throughout tumor growth. The cell loss factor, which indicates the cell loss rate as a fraction of the cell production rate, was 15.8% in small 10-day-old sarcomas and 48% in 20-day-old tumors. The proliferation kinetics of the viral-induced renal sarcoma corresponded with previous findings in other tumors in most respects; however, the constancy of the PF during tumor growth was a pecularity of the renal sarcoma.

0238 CHANGES IN CHROMATIN MORPHOLOGY AFTER INFECTION OF MOUSE EMBRYO FIBROBLASTS WITH POLYOMA VIRUS, DETECTED BY IMAGE ANALYSIS. (Eng.) Rowinski, J. (Wistar Inst. Anat. Biol., Philadelphia, Pa.); Sawicki, W.; Swenson, R.; Koprowski, H. *Acta Cytol. (Baltimore)* 19(2):136-141; 1975.

Nuclear chromatin properties in control, Simian virus 40 (SV40)-infected and polyoma-infected cultures of ICR Swiss mouse embryo fibroblasts were studied using quantitative image analysis. Cells were incubated with either hamster anti-SV40-tumor (T) serum or hamster anti-polyoma-virus (V) serum for 30 min, washed in phosphate buffered saline and incubated with FITC-labeled rabbit antihamster globulin for 30 min. The cells were fixed, Feulgen stained, and examined for automated image analysis of cell nuclei using a 720D densitometer module. In cultures exposed to SV40, 68% of the cells were positive for SV40-T-antigen and all were negative for polyoma-V-antigen; in cultures exposed to polyoma virus, 79% of the cells were positive for polyoma V-antigen, and all were negative for SV40-T-antigen. In control (mock-infected) cultures all cells were negative for both antigens. Distribution of Feulgen-DNA content per nucleus was similar in all three groups of cultures. No differences in nuclear area, mean optical density of chromatin and area of chromatin at different optical density thresholds were measurable between control and SV40-infected fibroblasts. Polyoma infected fibroblasts had smaller nuclei, more dense and less homogeneous chromatin than non-infected cells. The changes of chromatin in polyoma-infected cells occurred in G_1, middle S and G_2 nuclei. The results are interpreted as an early manifestation of the cytopathic effect of polyoma virus.

0239 THE CYTODIAGNOSIS OF HUMAN POLYOMAVIRUS INFECTION. (Eng.) Coleman, D. V. (St. Mary's Hosp. Med. Sch., London, England). *Acta Cytol. (Baltimore)* 19(2):93-96; 1975.

The cytodiagnosis of human polyomavirus infection was made in cytologic preparations of urine in a patient. The inclusion-bearing cells were filtered through the urine and examined by light microscopy. Each cell contained a large single basophilic homogeneous intranuclear inclusion body and the nuclear membrane appeared thickened by a heavy deposition of chromatin on the inner surface. In a few cells the inclusion was separated from the nuclear membrane by a clearly defined halo and this conferred on the cells the bird's eye appearance which is generally associated with cytomegalovirus infection. Cells pelleted at low speed from the urine were observed with the electron microscope. Virus particles which were morphologically identified with members of the polyoma subgroup of the papovaviruses were seen in the nuclei of the exfoliated cells. Based on this history and a cytologic study of patients with functioning renal allografts, it is concluded that the presence of numerous cells bearing intranuclear inclusions in the urine is strongly suggestive of infection with human polyomavirus. However, a cytodiagnosis of the inclusion bodies in the urine should always be confirmed by electron microscopy and virus isolation studies to ascertain the specific virus involved.

0240 SURFACE CARBOHYDRATES OF HAMSTER FIBROBLASTS. I. CHEMICAL CHARACTERIZATION OF SURFACE-LABELED GLYCOSPHINGOLIPIDS AND A SPECIFIC CERAMIDE TETRASACCHARIDE FOR TRANSFORMANTS. *(Eng.)* Gahmberg, C. G. (Sch. Public Health, Univ. Washington, Seattle); Hakomori, S.-I. *J. Biol. Chem.* 250 (7):2438-2446; 1975.

The surface glycolipids of hamster NIL 2K fibroblasts and polyoma virus-transformed NIL cells (NILpy) were fractionated and chemically characterized. Galactose oxidase was used to oxidize the terminal galactosyl and *N*-acetylgalactosaminyl residues of the surface-exposed glycolipids and glycoproteins to their corresponding C6-aldehydes; the latter were then reduced by tritiated borohydride. The glycolipids were characterized following methanolization, methylation, or enzymatic degradation and their immunological reactivities were then determined. The neutral glycosphingolipids of the NIL 2K cells were characterized as follows: glucosylceramide, lactosylceramide (βGal1→4Glc→Cer), a digalactosylceramide (αGal1→4βGal→Cer), a trihexosylceramide (αGal1→4βGal1→4Glc→Cer), two kinds of ceramide tetrasaccharides (A: αGalNAc1→3βGalNAc1→3αGal1→4βGal1→1Cer, a new type of Forssman active glycolipid; and B: globoside, βGalNAc1→3αGal1→4βGal1→4βGlc→Cer), and a ceramide pentasaccharide having a classical structure for Forssman antigen (αGalNAc1→3βGalNAc1→3αGal1→4βGal1→4Glc→Cer). The neutral glycosphingolipids of the NILpy cells had an additional ceramide tetrasaccharide which was absent from the NIL 2K cells. The structure of this specific glycolipid identified it as lacto-*N*-neotetraosylceramide (βGal1→4βGlcNAc1→3βGal1→4Glc→Cer). The quantities of the ceramide tetra- and pentasaccharides in the NILpy cells were much lower than in the NIL 2K cells. Following galactose oxidase labeling, the specific activities of the label in the glycolipid of the NILpy cells were much greater than in the NIL cells. The surface label in the glycolipids was cell cycle-dependent in the NIL cells, and a remarkable exposure of a galactosyl residue of a ceramide tetrasaccharide was demonstrated only on the NILpy cells, due to the presence of lacto-*N*-neotetraosylceramide.

0241 POLYPEPTIDE COMPOSITION OF CELL MEMBRANES FROM CHICK EMBRYO FIBROBLASTS TRANSFORMED BY ROUS SARCOMA VIRUS. (Eng.) Marciani, D. J. (Natl. Cancer Inst., Bethesda, Md. 20014); Bader, J. P. *Biochim. Biophys. Acta* 401(3):386-398; 1975.

Chick embryo fibroblasts were transformed by the Bryan high-titer strain of Rous sarcoma virus (RSV-BH), or a mutant (RSV-BH-Ta) inducing temperature-dependent transformation. Surface membranes from normal and transformed cells were isolated as membrane vesicles by differential centrifugation, and as cell ghosts after $ZnCl_2$ treatment and separation in an aqueous two-phase system. These preparations were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate or phenol/urea/acetic acid. In general a greater resolution of individual bands was found in gels containing phenol/urea/acetic acid, which separates polypeptides on the bases of size and charge. Electrophoresis of preparations from nontransformed cells showed that two polypeptides (molecular wt 200,000 and 250,000) found in cell ghosts were missing in membrane vesicles. In cell ghosts, transformation by RSV-BH resulted in a significant decrease of the 250,000 molecular wt complex. Also a polypeptide (molecular wt 73,000) prominent in membrane vesicles from nontransformed cells was decreased in transformed cells. Surfaces from cells transformed by RSV-BH-Ta at 37 C presented patterns similar to those for RSV-BH infected cells. Shifting these cells to 41 C resulted in an increase in the 250,000 molecular wt complex, although the amount of this protein(s) never reached that found in noninfected cells. Inhibitors of RNA and protein synthesis (actinomycin D and cycloheximide, resp.) failed to block the morphological changes occurring in RSV-BH-Ta cells after temperature shifts from 41 C to 37 C or vice-versa. The same inhibitors caused a reduction in the levels of the 250,000 molecular wt complex at both temperatures. These data indicate that these large membrane-associated polypeptides play little or no role in the morphological changes associated with transformation and its reversal.

0242 GENETIC CONTROL OF SUSCEPTIBILITY OF MICE TO ROUS SARCOMA VIRUS TUMORIGENESIS: I. TUMOR INCIDENCE IN INBRED STRAINS AND F_1 HYBRIDS. (Eng.) Whitmore, A. C. (Sch. Medicine, Univ. North Carolina, Chapel Hill, N.C.); Haughton, G. *Immunogenetics* 2(4):379-388; 1975.

The genetic factors affecting the incidence of Rous sarcoma virus (RSV)-induced tumors in mice were investigated. Newborn mice of 11 different inbred strains of three separate genetic backgrounds [C57BL/10ScSn mice and their congenic resistant partners B10.A, B10.D2, B10.129(21M), and B10.C(47N); A/WySn mice and their congenic resistant partners A.BY, A.SW, and A.CA; CBA/J mice; and CBA-T6T6/J mice] were injected with fresh chicken Rous sarcoma suspension by 24 hr after birth. Living chicken tumor cells were necessary for the production of primary Rous sarcoma in mice; the injection of cell-free virus particles isolated from tissue culture supernatants of chicken embryo fibroblasts transformed by RSV was not successful in producing mouse tumors. In the mice with C57BL/10 background, there was no significant difference between the susceptibility of $H\text{-}2^b$ and $H\text{-}2^a$ strains, but the $H\text{-}2^d$ haplotype of B10.D2 conferred highly significant resistance to tumors. Tumor incidence in two non-*H-2* congenic strains, B10.C (47N) and B10.129(21M), was not significantly different from the parent strain. Tumor incidence in the hybrid (C57BL/10 x B10.D2)F_1 was not significantly different from the C57BL/10 parent, indicating that susceptibility is dominant. In mice with the A background, there was a lower incidence of Rous sarcoma in $H\text{-}2^b$ mice than in $H\text{-}2^a$ (34.2 *versus* 60.1%). A.CA strain did not differ from the A mice in susceptibility, whereas the $H\text{-}2^s$ haplotype of A.SW was associated with a highly significant resistance to tumor formation. The F_1 hybrid of A/WynSn $H\text{-}2^a$ and A.SW ($H\text{-}2^s$) had intermediate susceptibility. A low incidence hybrid (A.SW x B10.D2)F_1, was produced by two low-incidence strains indicating that C57BL/10-B10.D2 and A-A.SW differences are probably due to the same gene. Tumor incidence in hybrids produced between the high-incidence CBA strain and two low-incidence strains and an intermediate strain did not differ significantly from the non-CBA parent. This implies that a different gene(s) on the CBA background manifests recessive susceptibility. These results are compared with other studies on *H-2*-associated genes affecting murine viral oncogenesis.

0243 ALTERATIONS IN SURFACE PROTEINS IN CHICKEN CELLS TRANSFORMED BY TEMPERATURE-SENSITIVE MUTANTS OF ROUS SARCOMA VIRUS. *(Eng.)* Hynes, R. O. (Imp. Cancer Res. Fund Lab., London, England); Wyke, J. A. *Virology* 64(2):492-504; 1975.

Temperature-sensitive (*ts*) mutants of Rous sarcoma virus (RSV) and lactoperoxidase-catalyzed iodination were used to study the changes in surface proteins associated with viral transformation. Secondary chick embryo fibroblast (CEF) cultures were infected with mutants of the Prague strain of RSV, subgroup A, and were incubated at the permissive temperature (35 C) until the first clear signs of cell transformation at 3-4 days postinfection. The cultures were then maintained until the seventh day postinfection at the non-permissive temperature (41 C), after which they were transferred to Nunc plastic petri dishes and maintained for 36-48 hr at 35 or 41 C. All cell monolayers were incubated with [^{125}I] iodide, lactoperoxidase and glucose oxidase for 10 min at room temperature. The cultures were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and determinations were made of the rate of 2-deoxy-D-glucose uptake and the rate of protein synthesis after incubation with [^{35}S] methionine. A large external transformation-sensitive (LETS) protein was detected by lactoperoxidase-catalyzed iodination on normal CEF but was reduced on the RSV-transformed cells. The LETS protein was temperature sensitive in the *ts* RSV-infected CEF: during reversion to normality on temperature shift-up, the protein appeared at the surface quite rapidly and in parallel with the morphological changes due to transformation, although the change was less rapid than that of glucose transport rate; on transformation during temperature shift-down, the disappearance of the LETS

protein lagged behind the morphological change. Cycloheximide did not inhibit the reappearance of the iodinatable LETS protein on shift-up, which suggests that the protein is synthesized in the transformed cells although it is not present at the surface. The data suggest an increased rate of turnover of the LETS protein after transformation, possibly due to the involvement of proteases released by the transformation process.

0244 TEMPERATURE-SENSITIVE EXPRESSION OF DIFFERENTIATION IN TRANSFORMED MYOBLASTS. (Eng.) Fiszman, M. Y. (Dep. Biol., Massachusetts Inst. Technol., Cambridge); Fuchs, P. *Nature* 254(5499): 429-431; 1975.

The *in vitro* transformation of chick embryo myogenic cells with a temperature-sensitive mutant of Rous sarcoma virus (RSV) is reported. Chick myoblasts derived from thigh muscles of 11-day-old chick embryos were infected with the Schmidt-Ruppin ts68 strain of RSV. In mock-infected chick embryo myoblasts, after five days in culture, most of the mononucleated cells were found replaced by long myotubes, each containing numerous nuclei. However, in the RSV-infected cultures, the number of myotubes found was reduced, and many aggregates of rounded cells, presumably transformed, were found. A quantitative study revealed a change in the overall composition of the population after a shift from 36 C (permissive temperature) to 41 C (nonpermissive temperature); whereas at 36 C, 99% of the cells remained mononucleated; 60% became polynucleated at 41 C. RSV-transformed myoblasts could be isolated, and after serial passage, these myoblasts were prevented from further differentiation. The function was not lost, however, because a shift up to a nonpermissive temperature again revealed differentiation to form myotubes. The cells which did not fuse at 41 C could have been transformed fibroblasts, or transformed myoblasts which had definitively lost their ability to differentiate. A striking difference was found between the myotubes appearing after infection and those formed after the shift to 41 C: the latter were found very stable and remained as long as myotubes in an uninfected culture. The results indicate that it is possible to isolate transformed myoblasts in which the expression of differentiation is under viral gene control.

0245 RNA-DIRECTED DNA SYNTHESIS BY THE DNA POLYMERASE OF ROUS SARCOMA VIRUS: STRUCTURAL AND FUNCTIONAL IDENTIFICATION OF 4S PRIMER RNA IN UNINFECTED CELLS. *(Eng.)* Faras, A. J. (Univ. Michigan Med. Sch., Ann Arbor); Dibble, N. A. *Proc. Natl. Acad. Sci. USA* 72(3):859-863; 1975.

The purification of a 4S RNA from uninfected avian cells which is structurally similar to the Rous sarcoma virus (RSV) 4S RNA primer is reported. Labeled and unlabeled primer was purified from the 70S RNA complex of RSV by a differential melt procedure or two-dimensional electrophoresis on polyacrylamide gels. Labeled and unlabeled 4S RNA from chick and duck embryos, mouse liver, human spleen, and HeLa cells was obtained by rate-zonal sedimentation, salt fractionation, treatment with RNase-free DNase, and/or DEAE-cellulose column chromatography. The 4S RNA was studied by two-dimensional polyacrylamide gel and paper electrophoresis and its ability to actively participate as primer in the initiation of RNA-directed DNA synthesis by the RSV DNA polymerase was determined. The 4S RNA from uninfected chick, duck, mouse, and human cells was able to restore template activity to the viral RNA genome from which all primer had been removed; similar primer activity appeared to be low or absent yeast eukaryotes and *E. coli* prokaryotes. Two-dimensional paper electrophoresis of oligonucleotides obtained from a T1 ribonuclease digest of the 4S RNA and RSV primer showed structural similarities between the two molecules. It is concluded that the RSV DNA polymerase can utilize a host cell molecule as primer for the initiation of RNA-directed DNA synthesis *in vitro*.

0246 DECREASE IN MEMBRANE-ASSOCIATED ACTIN OF FIBROBLASTS AFTER TRANSFORMATION BY ROUS SARCOMA VIRUS. (Eng.) Wickus, G. (Biol. Dep., Massachusetts Inst. Technol., Cambridge); Gruenstein, E.; Robbins, P. W.; Rich, A. *Proc. Natl. Acad. Sci. USA* 72(2):746-749; 1975.

A comparison was made of the actin content of membranes prepared from chick embryo fibroblasts before and after transformation of the cells by Rous sarcoma virus. Actin content was measured on polyacrylamide gels and was identified by tryptic peptide mapping. After transformation, the amount of actin in the membranes was decreased by 30-50%, but the total actin content of the cells was unchanged. This decrease was not believed to be due to the infection *per se*, as infection by a temperature-sensitive strain of Rous sarcoma virus (TS 68) decreased the actin content of membranes only at the permissive temperature. The effect of temperature on membrane actin reduction was examined. A shift from the nonpermissive (41 C) to the permissive (36 C) temperature resulted in an increase in the percentage of total protein synthesis devoted to actin production. Assuming no gross change in the isolation and recovery of plasma membranes associated with transformation, the authors conclude that in transformation there is a redistribution of cellular actin from the plasma membrane, but no decrease in total cellular actin.

0247 ENHANCEMENT OF SENDAI VIRUS-MEDIATED FUSION AND HYBRIDIZATION OF LYMPHOID CELLS BY ADDITION OF PHYTOHEMAGGLUTININ. (Eng.) Yoshida, M. C. (Faculty of Science, Hokkaido Univ., Sapporo, Japan); Ikeuchi, T. *Proc. Jpn. Acad.* 51(2):126-129; 1975.

Phytohemagglutinin (PHA) was tested for its ability to enhance Sendai virus-mediated cell fusion and interspecific hybridization in lymphoid cell lines. The lymphoid cell lines used included P3HR-1, a subclone originating from a Burkitt lymphoma; NC-37-TG, a 6-thioguanine (TG)-resistant mutant clone isolated from normal peripheral blood; and D1-TG, a TG-resistant mutant clone isolated from the Dunning rat leukemia. Mouse Cl 1D cells, resistant to 5-bromodeoxy-

uridine and deficient in thymidine kinase, were used as the monolayer culture. PHA, added after treatment of cell suspensions with UV-inactiviated Sendai virus (800 hemagglutination U/ml), significantly increased the percentage of multinucleate cells formed during fusion. At 75 μg/ml, PHA treatment produced fusion indices of 22.9% in D1-TG, 21.5% in NC-37-TG, and 21.2% in Cl 1D compared with control values of 2.5%, 1.9%, and 15.4%, respectively. The yield of multinucleate cells in P3HR-1 was 6.7% at 15 μg/ml PHA *versus* 1.9% in control cells. Heterokaryon formation, studied in D1-TG x Cl 1D and NC-37-TG x Cl 1D, was enhanced by PHA in a dose-dependent manner. The number of hybrid colonies formed in experiments with D1-TG x Cl 1D and NC-37-TG x Cl 1D increased with increasing concentrations of PHA, reaching a plateau at 75 μg/ml. Karyotype analysis confirmed that the hybrid cells contained most chromosomes derived from both parental cells. It is suggested that the striking effects of PHA on cell fusion may be due to cellular agglutination. This may facilitate the initiation of membrane fusion by increasing cell-to-cell contact among viral adsorbed cells.

0248 FATE OF PARENTAL SIMIAN VIRUS 40 DNA IN PERMISSIVE MONKEY KIDNEY CELLS. (Eng.)
Howe, C. C. (Wistar Inst. Anat. Biol., Philadelphia, Pa.); Tan, K. B.; Sokol, F. *J. Gen. Virol.* 27(1): 11-24; 1975.

The fate of intracellular parental simian virus 40 (SV40) DNA in African green monkey kidney cells was investigated by infecting the cells with purified virus labeled with [^{3}H]-thymidine. 5-Bromodeoxyuridine (200 μg/ml) was added to the cells at 2 hr after infection to label virus progeny DNA. At 72 hr postinfection the cells were harvested and virus DNA was extracted and fractionated by isopycnic sedimentation in CsCl solution. Approximately 41% of the cell-associated parental label was released into the culture medium; more than 60% of this radioactivity was acid soluble indicating that degradation of input SV40 DNA had occurred. Approximately 23 and 1.2% of the parental label was recovered in the low and high molecular weight DNA fractions, resp. The following DNAs with characteristic densities were found: light (LL), 1.70 g/ml; hybrid (HL), 1.75 g/ml and heavy (HH), 1.80 g/ml. Of the total cell-associated [^{3}H]-radioactivity more than 90% was recovered in unreplicated parental DNA (LL DNA), about 2% was recovered in the HL DNA and about 0.7% was associated with the HH DNA. The unreplicated parental DNA was present as uncoated intact DNA complexed with proteins present in the infected cell. The HL DNA contains one light parental and one heavy progeny DNA strand. The results confirm previous observations that SV40 DNA replicates by a semiconservative mechanism.

0249 CHROMOSOME ANALYSIS OF A SIMIAN VIRUS 40-TRANSFORMED MOUSE CELL LINE AND TWO VARIANT SUBLINES THAT ARE RESISTANT TO CYTOCHALASIN B[1]. *(Eng.)* Kelly, F. (Cold Spring Harbor Lab., N.Y.). *Cancer Res.* 35(5):1210-1213; 1975.

The acetic-saline-Giemsa banding technique was used to analyze the chromosomes of the simian virus 40 (SV40)-transformed mouse cell line, SVT2, and two Cytochalasin B-resistant (CBR) sublines derived from SVT2 (CBR-1 and CBR-2). The chromosomes were counted on photographs of well-spread unbroken metaphase plates and scored as telocentric or biarmed, and individual chromosomes were identified by their banding pattern. The mean number of chromosomes per SVT2 cell was 40, with a mean of three biarmed chromosomes. Most of the elements in these cells were identified as normal mouse chromosomes and two copies of most autosomes were usually present. Chromosome 3 was, however, trisomic in most cells and chromosome 19 showed great variability, trisomy for this chromosome being frequently encountered. In addition, two copies of chromosome X were present in 75% of the cells and no Y chromosomes were detected. The CBR lines differed from the parental SVT2 cells in the total number of chromosomes, the proportion of biarmed chromosomes, and the chromosome distribution: the CBR-2 cells contained 40-42 chromosomes of which 0-2 were biarmed; and the CBR-1 cells contained 36-41 chromosomes, of which 0-5 were biarmed. All of the CBR-1 cells contained three of the marker chromosomes found in the SVT2 cells (1/3, 14/14, and 19/M1) as well as two new minute chromosomes not found in SVT2. Except for chromosome 19, all autosomes in CBR-1 and CBR-2 were diploid; in each case, there was only one X chromosome per cell. The CBR-2 cells contained only the 14/14 marker plus a new marker, M2. In general, the two CBR sublines contained one less chromosome arm than the SVT2 cells. SVT2, other near-diploid SV40-transformed cell lines, and variants derived from them provide a favorable system for studying specific interactions between the transforming virus and the host chromosome.

0250 EFFECT OF HOST AGE, VIRUS DOSE, AND ROUTE OF INOCULATION ON TUMOR INCIDENCE, LATENCY, AND MORPHOLOGY IN SYRIAN HAMSTERS INOCULATED INTRAVENOUSLY WITH ONCOGENIC DNA SIMIAN VIRUS 40. (Eng.)
Diamandopoulos, G. T. (Harvard Medical Sch., Boston, Mass. 02115); McLane, M.-F. *J. Natl. Cancer Inst.* 55(2):479-482; 1975.

A study was undertaken to determine if the age of the animals at the time of virus exposure, the dose of the virus and the route of virus inoculation could influence the incidence latency and morphologic variability of neoplasms. Three-wk-old to 12-mo-old male Syrian hamsters were inoculated iv with $10^{8.5}$ median tissue culture infective dose of simian virus 40 (SV40). Three-wk-old hamsters were similarly inoculated with aliquots of SV40 of progressively decreasing titers. The tumor incidence and, to a lesser extent, the tumor latency were directly dependent on the age of the animals at the time of virus exposure and on the dose of the virus. However, this age-dose dependence was not of the magnitude usually observed in hamsters inoculated with SV40 sc or im. Moreover, the wide morphologic spectrum of neoplasms induced, i.e., lymphosarcoma, reticulum cell sarcoma, and osteogenic sarcoma, by iv route of inoculation, contrasted sharply with the anaplastic and spindle-cell sarcomas which were the only types of malignant tumors resulting when other routes were used.

0251 MOBILITY OF NORMAL AND VIRUS-TRANSFORMED CELLS IN CELLULAR AGGREGATES. (Eng.) Gershman, H. (Case Western Reserve Univ. Medical Sch., Cleveland, Ohio 44106); Drumm, J. *J. Cell Biol.* 67(2/Part 1):419-435; 1975.

Studies were undertaken to resolve the discrepancies between the data on chick embryonic tissue cells and to investigate the mobility of normal and malignant fibroblasts in cellular aggregates. The mobility of embryonic chick cells and cells of four established cell lines was examined in cellular aggregates. This was done by preparing aggregates of unlabeled cells and allowing cells of the same type, but prelabeled with [^{3}H]thymidine, to adhere to the surface of the aggregates. After 2-½ days in agitated liquid culture the positions of the labeled cells within the aggregates were determined by autoradiographic techniques. Since the labeled and unlabeled cells were otherwise identical, the degree of penetration of the labeled cells into the aggregates was taken as a measure of the mixing or mobility of cells in the aggregate. With this procedure, embryonic chick liver, heart, and neural retina cells were found to move an average of 2.12, 2.68, and 4.00 cell diameters inward, respectively. Mouse fibroblast BALB/c 3T3 cells moved an average of 1.13 cell diameters inward, while Simian virus 40 (SV40)-transformed BALB/c 3T3 cells moved as much as 8.80 cell diameters inward, indicating that cells of the malignant SV40-transformed line were considerably more mobile than the corresponding nonmalignant 3T3 cells. In contrast, cells of the hamster fibroblast line NIL B moved 4.17 cell diameters in 2-½ days, while SV40-transformed NIL B cells moved 3.00 cell diameters in the same time. It was therefore concluded that infection with oncogenic viruses does not necessarily result in increased cellular mobility.

0252 BIOCHEMICAL METHOD FOR MAPPING MUTATIONAL ALTERATIONS IN DNA WITH S1 NUCLEASE: THE LOCATION OF DELETIONS AND TEMPERATURE-SENSITIVE MUTATIONS IN SIMIAN VIRUS 40. *(Eng.)* Shenk, T. E. (Stanford Univ. Sch. Med., Calif.); Rhodes, C; Rigby, P. W. J.; Berg, P. *Proc. Natl. Acad. Sci. USA* 72(3):989-993; 1975.

S1 nuclease, a single-strand-specific nuclease, was used to map the location of mutational alterations in simian virus 40 (SV40) DNA. To map a deletion, a mixture of unit length, linear DNA (prepared from the SV40 deletion mutant) was denatured and annealed along with its wild type parent to form heteroduplexes. The S1 nuclease then cut such heteroduplexes at the nonbase-paired region to produce fragments whose length corresponded to the position of the deletion. Specific fragments were also produced with S1 nuclease, which cleaved a heteroduplex formed from the DNAs for SV40 temperature-sensitive mutants and either their revertants or wild-type parents. In this way, the positions of the nonhomologies between the DNAs were determined. Using these procedures, deletions of between 32 and 190 base pairs, which are at or below the limit of detectability by conventional electron microscopic analysis, were located. The results with the temperature-sensitive mutants and revertants of SV40 further suggest that S1 nuclease can detect and map single base changes as well; this conclusion is still provisional, however, because it is unproven that the temperature-sensitive DNAs differ from their revertant or wild-type DNAs by only single bases at the sites where S1 nuclease acts. Despite several shortcomings, S1 nuclease mapping provides an approach to genetic mapping in systems where genetic recombination is lacking or difficult to measure, and it enables one to locate genotypic alterations that cause no detectable phenotypic changes. The ability of S1 nucleases to cleave a duplex DNA at a mismatch may also make it possible to isolate discrete segments of a genome if that segment can be bounded by small deletions.

0253 PATTERNS OF ORGANIZATION OF ACTIN AND MYOSIN IN NORMAL AND TRANSFORMED CULTURED CELLS. (Eng.) Pollack, R. (Cold Spring Harbor Lab., N.Y. 11724); Osborn, M.; Weber, K. *Proc. Natl. Acad. Sci. USA* 72(3):994-998; 1975.

Actin and myosin in normal and transformed cell cultures were examined by immunofluorescence using antibody directed against actin and antibody directed against myosin. Immunofluorescence revealed the position of actin and myosin within a series of normal, simian virus 40 (SV40)-transformed and revertant cells of rat and mouse origin. Cell lines included: normal mouse 3T3, SV101 (a 3T3 line transformed by SV40), F1SV101 (a density-sensitive revertant of SV101), A_γ and LS (serum-sensitive revertants of SV101), primary rat embryo (RE), SV40-transformed RE (including SVRE 9, SVRE 12, and WT4), and *tsA* SV40-transformed RE (*tsA*28-3). The relationship between transformation and the loss of actin-containing sheaths was correlated with anchorage dependence, cell density, and growth in low serum concentration. Loss of actin-containing sheaths and anchorage occurred in the following cell lines: SV101, A_γ, LS, SVRE 9, WT4, and *tsA*28-3. Data obtained with actin antibody showed that different cells display different distributions of actin-containing sheaths. SV40 transformation of 3T3 cells is accompanied by decreased actin-containing sheaths, and the reversion of SV101 to a flat revertant line (F1SV101) shows increased actin-containing sheaths. The results of myosin antibody on various cell lines were qualitatively similar to those obtained with actin antibody. Of the three parameters studied, only anchorage-dependance is correlated with the loss of actin-containing sheaths.

0254 NON-SPECIFIC TERMINATION OF SIMIAN VIRUS 40 DNA REPLICATION. (Eng.) Lai, C.-J. (Johns Hopkins Univ. Sch. Medicine, Baltimore, Md. 21205); Nathans, D. *J. Mol. Biol.* 97(1):113-118; 1975.

The termination site of bidirectionally replicating simian virus 40 DNA molecules was examined with mutants which contain a deletion in either replicating arm. African green monkey kidney cells (BSC-1 line) were infected with the deletion mutants at a multiplicy of infection of 0.5 complementing U/cell. Pulse-label distribution in newly completed viral DNA and electron microscopic measurement of replicating molecules were consistent with a shift of replication terminus in each mutant genome to a site

opposite the origin of replication. Based on these results, it is concluded that SV40 DNA replication terminates wherever two growing forks meet and involves no specific nucleotide sequence signal.

0255 TRANSFORMATION OF PRIMARY RAT KIDNEY CELLS BY FRAGMENTS OF SIMIAN VIRUS 40 DNA. (Eng.) Abrahams, P. J. (Lab. Physiological Chemistry, State Univ. Leiden, Netherlands); Mulder, C.; van de Voorde, A.; Warnaar, S. O.; van der Eb, A. J. *J. Virol.* 16(4):818-823; 1975.

Linear simian virus 40 (SV 40) DNA molecules of genome length and DNA fragments smaller than genome length were prepared with restriction endonucleases and tested for transforming activity on primary cultures of baby rat kidney cells. The linear molecules of genome length (prepared with endonucleases R·*Eco*RI, R·*Bam*HI, and R·*Hpa*II or R·*Hap*II), a 74% fragment (*Eco*RI/*Hpa*II or *Hap*II-A), and a 59% fragment (*Bam*HI/*Hap*II-A) could all transform rat kidney cells with the same efficiency as circular SV40 DNA. All transformed lines tested contained the SV40-specific T-antigen in 90 to 100% of the cells, which was taken as evidence that the transformation was SV40 specific. The DNA fragments with transforming activity contained the entire early region of SV40 DNA. Endo R·*Hpa*I, which introduced one break in the early region, apparently inactiviated the transforming capacity of SV40 DNA, since no transformation was observed with any of the three *Hpa*I fragments tested. Attempts were made to rescue infectious virus from some of the transformed lines by fusion with permissive BSC-1 cells. Infectious virus was only recovered from the cells transformed by circular form I DNA. No infectious virus could be isolated from any of the other types of transformed cells.

0256 SIMIAN VIRUS 40 INTEGRATION SITES IN THE GENOME OF VIRUS-TRANSFORMED MOUSE CELLS. (Eng.) Prasad, I. (New York Univ. Sch. Medicine, New York, N.Y. 10016); Zouzias, D.; Basilico, C. *J. Virol.* 16(4):897-904; 1975.

To gain information on the specificity of simian virus 40 (SV40) integration in the genome of transformed cells, mouse 3T3 cells were transformed by a temperature-sensitive (ts) SV40 mutant, using high muliplicity of infection (MOI). Transformed cells were superinfected with wild-type (wt) virus at high MOI. Clones were isolated and fused with permissive BSC-1 cells to promote virus rescue. All rescued viruses were of the ts type only. When the high-MOI transformants were infected with ^{3}H-labeled wt SV40, the amount of radioactivity associated with their nuclear fraction was found to be similar to that of 3T3 cells. 3T3 cells were then transformed by ts SV40 and low MOI and superinfected by wt virus at high MOI. Upon fusion with BSC-1 cells, most clones produced both ts and wt virus. These results suggest that the number of stable SV40 integration sites in the 3T3 genome is limited, since they can be saturated by transformation at high MOI. When the MOI is low, the sites are not saturated and a subsequent infection can lead to integration.

0257 FATTY ACID CHAIN FLEXIBILITY IN THE MEMBRANES OF NORMAL AND TRANSFORMED FIBROBLASTS. (Eng.) Gaffney, B. J. (Dep. Chem., Johns Hopkins Univ., Baltimore, Md.). *Proc. Natl. Acad. Sci. USA* 72(2):664-668; 1975.

A comparison of the flexibility of lipid acyl chains in intact cell membranes of normal and several varieties of transformed 3T3 mouse fibroblasts was made. Lipid chain flexibility was measured by paramagnetic spin labels. In contrast to a previously published report, no significant differences in lipid chain flexibility were discovered between normal and transformed mouse fibroblasts. The results were compared with earlier spin label measurements of normal and Rous sarcoma virus-transformed chick embryo fibroblasts in which similar results had been obtained. Because differential mobility of glycoproteins for normal and transformed cells has been demonstrated or inferred from differential agglutinability, the suggestion that differential lipid motion could be correlated with differential glycoprotein mobility was examined. The authors conclude that no basis was found for making this correlation.

0258 GENETIC ANALYSIS OF ADENOVIRUS TYPE 2: II. PRELIMINARY PHENOTYPIC CHARACTERIZATION OF TEMPERATURE-SENSITIVE MUTANTS. (Eng.) Weber, J. (Departement de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada); Begin, M.; Khittoo, G. *J. Virol.* 15(5):1049-1056; 1975.

0259 RETINAL TUMOR INDUCTION BY OCULAR INOCULATION OF HUMAN ADENOVIRUS IN 3-DAY-OLD RATS. (Eng.) Mukai, N. (Retina Found., Boston, Mass.); Murao, T. *J. Neuropathol. Exp. Neurol.* 34(1):28-35; 1975.

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0261 TRANSFORMATION OF HUMAN EMBRYONIC FIBROBLAST CELLS WITH DYE-LIGHT INACTIVATED HERPES VIRUS TYPE 2 [abstract]. (Eng.) Kucera, L. S. (Bowman Gray Sch. Med., Winston-Salem, N.C.); Gusdon, J. P., Jr. *Gynecol. Invest.* 6(1/2):42; 1975.

0262 POTENTIATION OF AN ADENOVIRUS-ASSOCIATED VIRUS BY HERPES SIMPLEX VIRUS TYPE-2-TRANSFORMED CELLS. (Eng.) Blacklow, N. R. (Boston Univ. Med. Cent., Mass.). *J. Natl. Cancer Inst.* 54(1):241-244; 1975.

0263 ISOLATION OF TYPE-C VIRUSES FROM THE ASIAN FERAL MOUSE *MUS MUSCULUS MOLOSSINUS*. (Eng.) Lieber, M. (Natl. Cancer Inst., Bethesda, Md.); Sherr, C.; Potter, M.; Todaro, G. *Int. J. Cancer* 15(2):211-220; 1975.

0264 TYPE-C VIRUS PARTICLES IN PLACENTA OF THE COTTONTOP MARMOSET (*SAGUINUS OEDIPUS*). (Eng.) Seman, G. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.); Levy, B. M.; Panigel, M.; Dmochowski, L. *J. Natl. Cancer Inst.* 54(1):251-252; 1975.

0265 HYDRODYNAMIC DIAMETERS OF RNA TUMOR VIRUSES. STUDIES BY LASER BEAT FREQUENCY LIGHT SCATTERING SPECTROSCOPY OF AVIAN MYELOBLASTOSIS AND RAUSCHER MURINE LEUKEMIA VIRUSES. (Eng.) Salmeen, I. (Sci. Res. Staff, Ford Mot. Co., Dearborn, Mich.); Rimai, L.; Liebes, L.; Rich, M. A.; McCormick, J. J. *Biochemistry* 14(1):134-141; 1975.

0266 VIRUS ENVELOPE MARKERS IN MAMMALIAN TROPISM OF AVIAN RNA TUMOR VIRUSES. (Eng.) Boettiger, D. (Imp. Cancer Res. Fund Labs., London, England); Love, D. N.; Weiss, R. A. *J. Virol.* 15(1):108-114; 1975.

0267 FRIEND LEUKEMIA: RAPID DEVELOPMENT OF ERYTHROPOIETIN-INDEPENDENT HEMATOPOIETIC PRECURSORS. (Eng.) Horoszewicz, J. S. (Dept. Med. Viral Oncol., Roswell Park Mem. Inst., Buffalo, N.Y.) Leong, S. S.; Carter, W. A. *J. Natl. Cancer Inst.* 54(1):265-267; 1975.

0268 ASSOCIATION OF FELINE LEUKEMIA VIRUS WITH LYMPHOSARCOMA AND OTHER DISORDERS IN THE CAT. (Eng.) Cotter, S. M. (Angell Mem. Anim. Hosp., Boston, Mass.), Hardy Jr., W. D.; Essex, M. *J. Am. Vet. Med. Assoc.* 166(5):449-454; 1975.

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0272 INDUCTION OF LEUKEMIA IN RAUSCHER VIRUS-RESISTANT MICE BY MIXED INFECTION WITH M ARTHRITIDIS AND RAUSCHER VIRUS. (Rus.) Kagan, G. Ia. (N. F. Gamaleya Inst. Epidemiol. Microbiol., Acad. Med. Sci. USSR, Moscow, USSR); Rakovskaia, I. V.; Morgunova, T. D.; Postnikova, Z. A. *Vopr. Virusol.* (2):171-176; 1975.

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0275 NEW MUTANT AND CONGENIC MOUSE STOCKS EXPRESSING THE MURINE LEUKEMIA VIRUS-ASSOCIATED THYMOCYTE SURFACE ANTIGEN G_{IX}. (Eng.) Stockert, E. (Memorial Sloan-Kettering Cancer Cent., New York, N.Y.); Boyse, E. A.; Obata, Y.; Ikeda, H.; Sarkar, N. H.; Hoffman, H. A. *J. Exp. Med.* 142(2): 512-517; 1975.

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0278 STUDY OF THE POPULATION COMPOSITION OF ROUS SARCOMA VIRUS CARR-SILBER STRAIN. (Rus.) Obukh, I. B. (N. F. Gamaleya Inst. Epidemiol. Microbiol., Acad. Med. Sci. USSR, Moscow, USSR); Kriukova, I. N. *Vopr. Virusol.* (2):163-167; 1975.

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0281 EFFECT OF SIMIAN VIRUS 40 SUBCUTANEOUS TUMORS ON CIRCULATING LIPIDS AND LIPOPROTEINS IN THE SYRIAN HAMSTER. (Eng.) Cox, R. A.;

(Health Cent., Inc., Golden Valley, Minn.); Gokcen, M. *J. Natl. Cancer Inst.* 54(2):379-386; 1975.

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0284 VIRUS-LIKE INCLUSION IN BASAL CELL CARCINOMA. (Ger.) Rupec, M. (Dermatologische Klinik der Philipps-Universitat, Deutschhausstrasse 9, D-355 Marburg an der Lahn, West Germany); Bruhl, R. *Experientia* 31(3):356-357; 1975.

0285 A SIMPLE CELL-SUSPENSION METHOD FOR TRANSPLANTATION OF V2 CARCINOMA. (Eng.) Berkowitz, D. M. (Harvard Med. Sch., Boston, Mass.); Alexander, L.; Hollenberg, N. K. *J. Natl. Cancer Inst.* 54(1):233-234; 1975.

See also:

* (Rev): 0007, 0008, 0012, 0032, 0034, 0035, 0036, 0037, 0038
* (Chem): 0080
* (Immun): 0286, 0291, 0292, 0300, 0301, 0302, 0303, 0312, 0313, 0317, 0321, 0342, 0357, 0368, 0376, 0377, 0382, 0383, 0390, 0391
* (Epid-Biom): 0505, 0512

0286 *IN VITRO* STUDIES ON THE CELLULAR IMMUNE RESPONSE OF TUMOR-BEARING MICE TO SV40-TRANSFORMED CELLS. (Eng.) Blasecki, J. W. (Tufts Univ. Sch. Med., Boston, Mass.); Tevethia, S. S. *J. Immunol.* 114(1):244-249; 1975.

Specific cell-mediated immunity to SV40 tumor-specific transplantation antigen in mice undergoing tumorigenesis by syngeneic SV40-transformed BALB/c cells was investigated by the macrophage migration inhibition and transplantation rejection tests. Specific cellular reactivity to SV40 tumor-specific transplantation antigen was observed as early as four days after tumor cell inoculation (1 x 10^5 viable tumor cells). This activity was not detectable during the later stages of tumor growth (ten days after inoculation) but was again demonstrable by macrophage migration inhibition two weeks after tumor excision. Addition of an equal number of nonreactive peritoneal exudate cells from tumor-bearing mice to peritoneal exudate cells from mice immune to SV40 tumor-specific transplantation antigen abrogated the reactivity of the latter to soluble, SV40 tumor-specific transplantation antigen. When lymphoid cells with blocking activity were cultured *in vitro* they not only lost their blocking capacity but also regained their reactivity to SV40 tumor-specific transplantation antigen (macrophage migration inhibition test). These findings suggest that tumor-bearing hosts possess lymphocytes specifically sensitized to the tumor-specific transplantation antigen of the tumor and that the specific reactivity of these cells can be regained after culture *in vitro*.

0287 THE IMMUNOBIOLOGY OF SKIN CANCER. (Eng.) Dellon, A. L. (Natl. Cancer Inst., Bethesda, Md.); Potvin, C.; Chretien, P. B.; Rogentine, C. N. *Plast. Reconstr. Surg.* 55(3):341-354; 1975.

Seventy-five skin cancer patients, 33 with squamous cell carcinoma (SCC), 26 with basal cell carcinoma (BCC), 13 with Bowen's disease (BD) and three with xeroderma pigmentosa (XP) were compared with 182 healthy Caucasian volunteers to determine differences between the two groups. T cell levels in peripheral blood were assayed by the spontaneous lymphocyte rosette assay and were found to be significantly lower in SCC and BCC patients than in controls. Patients with large tumors had lower T cell levels than those with small tumors, and the levels remained low in patients cured of large tumors while they returned to normal in patients cured of small tumors. T cell levels in patients with BD were also lower than normal. The degree of lymphocytic infiltration (LI) of tumors was determined in 21 patients and found to correlate with tumor size and T cell levels, in that small tumors had the greatest LI and T cell levels and large tumors had the lowest. Histocompatibility antigen typing was determined using a microcytotoxicity technique and the presence of HL-A antigens 1 and 8 was found to correlate with low T cell levels and a tendency towards large tumors. It is suggested that these findings may be useful in the clinical management of patients with skin cancer.

0288 IMMUNE COMPLEXES IN CANCER: DEMONSTRATION OF COMPLEXES IN MICE BEARING NEUROBLASTOMAS. (Eng.) Oldstone, M. B. A. (Scripps Clin. Res. Found., La Jolla, Calif.). *J. Natl. Cancer Inst.* 54(1):223-228; 1975.

Syngeneic neuroblastoma (C1300 line) cells were inoculated into A/J mice to study the formation of circulating immune complexes and deposits of IgG and C_3 in renal glomeruli. Circulating immune complexes were found in all mice, whereas only 50% demonstrated deposits of IgG and C_3 in the renal glomeruli. Histoimmunofluorescent studies showed the IgG and C_3 to be deposited in a granular pattern along the basement membranes and mesangia. Kidneys with IgG deposits were pooled and IgG was eluted and isolated for further study. The eluted IgG bound to antigens found on a C1300 section of tumor and C1300 cultured cells, but did not bind to surfaces of YCAB or MOPC 315 cells. Two to six μg of IgG were recovered/kidney. The results suggest that immune complex formation commonly accompanies tumors, and that tumor-specific antibodies or antigens are the components of the immune complexes found in this study.

0289 SURVIVAL AND DIFFERENTIATION OF CANINE MAMMARY TISSUES IN THE HAMSTER CHEEK POUCH. (Eng.) Richmond, R. E. (Dep. Anat., Univ. California, Davis); Faulkin, L. J., Jr. *Cancer Res.* 35(3):791-795; 1975.

Preneoplastic mammary nodules, neoplastic mammary tissues and normal mammary tissues from adult beagle dogs were transplanted into the cheek pouches of normal and immunosuppressed female golden hamsters in order to determine if heterotransplantation could be achieved with this model. Immunosuppression with prednisolone and antilymphocyte serum resulted in greater than 35- to 45-day survival of 78% of the mammary transplants. None of the transplants survived 40 days without immunosuppression. The morphology of surviving transplants remained similar to the canine donor tissue in 53 of 58 (91%) cases. The surviving transplants in all cases maintained the same size during the transplant period. Estrogen and progesterone treatments of host animals had no effect on tumor viability or size. The results indicate that heterotransplantation of normal, preneoplastic and neoplastic canine mammary tissues to immunosuppressed hamsters is possible, and may be a useful model to examine the growth and morphology of canine mammary tumors.

0290 IMMUNE COMPLEX DEPOSITION IN THYROID CARCINOMA ASSOCIATED WITH CHRONIC THYROIDITIS. (Eng.) Kalderone, A. E. (Roger Williams Gen. Hosp., Providence, R.I.); Bogaars, H. A.; Diamond, I. *Clin. Immunol. Immunopathol.* 4(1):101-107; 1975.

A case of multicentric papillary carcinoma of the left lobe of the thyroid, coexisting with focal chronic lymphocytic thyroiditis, is presented. Resected tumor tissue of a 25-yr-old woman was examined with light and electron microscopy. For immunological

tests, quick frozen tissue obtained at operation was sectioned, thawed, fixed in acetone, and treated with fluorescein-conjugated goat and rabbit antisera against human IgG, IgA, IgM, C3, fibrinogen, albumin, and thyroglobulin. Immune complex deposits were present in the follicular basement membrane of neoplastic follicles in area of heavy lymphocyte and plasma cell infiltration. These deposits correlated with positive immunofluorescent staining with anti-IgG, anti-C3, and anti-thyroglobulin. The results provide evidence that a humoral immune mechanism, other than cell-mediated immunity directed against thyroglobulin, may play a role in the pathogenesis of thyroiditis associated with carcinoma of the thyroid.

0291 ANTIBODY RESPONSE TO EPSTEIN-BARR VIRUS IN INFECTIOUS MONONUCLEOSIS. (Eng.) Nikoskelainen, J. (Dep. Virol., Univ. Turku, Finland); Hanninen, P. *Infect. Immun.* 11(1):42-51; 1975.

The antibody response to different Epstein-Barr virus (EBV) antigens was studied in 171 serum specimens from 58 patients with infectious mononucleosis (IM). All specimens were tested for fluorescent IgM and IgG antibodies to EBV using an indirect immunofluorescence technique; the sera were also tested for gel-precipitating (GP) and complement-fixing (CF) antibodies to EBV. All 58 patients had IgG and IgM antibodies to EBV, both of them developing rapidly in the course of the disease. The IgM antibodies disappeared rapidly after the onset of the disease, none being detectable after 12 weeks; in most patients, the IgG antibody levels remained fairly constant. Fifty patients had GP antibodies and these developed slowly in most cases; the mean titers kept rising for an average of ten weeks and were never observed to fall. The CF antibodies, detectable in 42 patients, also developed slowly and the titer kept rising for an average of ten weeks. All patients also had heterophil antibodies which developed soon after the onset of the illness, then disappeared rapidly; seven patients with IM had some rheumatoid factor-related activity in their sera. The results indicated that the most promising antibody assay in the diagnosis of recent IM infections is the EBV-specific IgM antibody assay, which allows diagnosis on the basis of one serum specimen. In cases where the acute-phase serum specimen is in remission, the diagnosis can later be made using the GP and CF techniques, the former appearing to be the most sensitive.

0292 ELEVATED IMMUNOFLUORESCENCE ANTIBODY TITERS TO SEVERAL HERPESVIRUSES IN BURKITT'S LYMPHOMA PATIENTS: ARE HIGH TITERS UNIQUE? (Eng.) Hilgers, F. (Netherlands Cancer Inst., Amsterdam); Dean, A. G.; de-Thé, G. *J. Natl. Cancer Inst.* 54(1):49-51; 1975.

An immunofluorescent technique was used to measure antibody titers to herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) in 16 Burkitt's lymphoma (BL) patients, 16 age-, sex- and locality-matched controls, and 136 relatives to determine if elevated antibody titers were unique to the BL patients. Antibody titers to EBV, CMV, and VZV were 4-fold higher in patients than in unrelated controls. EBV titers in BL patients' mothers were slightly higher than those in control mothers. No other familial differences were seen. The elevated titers to CMV and VZV were not due to serologic cross reactions. The results indicate that CMV and VZV titers are elevated in BL patients, as well as EBV titers.

0293 ALTERED ALLOTRANSPLANTABILITY OF BALB/c LEYDIG CELL TUMOR AFTER ORGAN CULTURE OR CELL SUSPENSION. (Eng.) Ninnemann, J. L. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Good, R. A. *Transplantation* 19(1):42-47; 1975.

Experiments directed toward confirming the allogeneic transplantability of testicular interstitial (Leydig) cell tumors after organ culture, extending tumor acceptance to other H-2 strain combinations, and determining lymphocyte cytotoxic response to organ-cultured and noncultured tumors are reported. Fresh noncultured tumor fragments grew progressively in BALB/c mice (the strain of origin) and in DBA/2 recipients. No tumor growth was observed in DBA/1, BUB/BnJ, C3H/HeJ, C57BL/6J, A/J, or D1.LP recipients. Tumor fragments which had been organ-cultured for 10 days grew in DBA/1 and BUB/BnJ recipients as well as in BALB/c and DBA/2 recipients but failed to grow progressively in the other strains. Transfer of tumor cell suspensions of 10^1, 10^2, and 10^3 cells failed to grow in any of the strains tested. Cell suspensions of 10^5 and 10^7 tumor cells produced the same growth pattern as organ-cultured tumor fragments. Lymphocyte cytotoxicity assays showed that while transplantability was altered by the organ culture process, tissue immunogenicity *in vitro* was unchanged. This work supplies evidence that the H-2^q locus is probably the locus of major importance in determining acceptance or rejection of tumor fragments after culture. D1.LP recipients, which are genetically identical to the DBA/1 animals except at the H-2 locus, consistently rejected culture explanted tumor. Rather than modifying cell surface antigens, it is possible that organ culture explantation serves to 1) allow free dispersal of the tumor cells which leads to progressive tumor growth, and/or 2) raise the ratio of dead to living cells in the transplanted fragments, which has been shown previously to direct host immune responses to enhancement.

0294 SUPPRESSION AND REGRESSION OF A TRANSPLANTED TUMOR IN THE GUINEA PIG COLON MEDIATED BY *MYCOBACTERIUM BOVIS*, STRAIN BCG. (Eng.) Harmel, R. P., Jr. (Public Health Serv., U.S. Dep. Health Educ. Welfare, Bethesda, Md.); Zbar, B.; Rapp, H. J. *J. Natl. Cancer Inst.* 54(2):515-517; 1975.

The effect of injections of strain BCG *Mycobacterium bovis* on growth of an immunogenic, well-differentiated hepatocarcinoma in guinea pig colon was determined. Line 16 of a hepatocarcinoma originally induced by diethylnitrosamine was maintained in strain-2 guinea pigs by injections of tumor cells into the colon wall. Normal animals died about 30

days after tumor implantation, when the tumors were 5-6 cm in diameter. If BCG was coinoculated along with the cancer cells, 13/15 animals survived for six months, indicating suppression of tumor growth. Injection of BCG into tumor masses seven days after implantation (when the tumors were 1-2 mm in diameter) caused 9/10 animals to survive for more than two months. Survival rates dropped if BCG was injected into nine- or ten-day-old tumors. Survivor animals coinoculated with BCG and tumor cells were subsequently treated with i.m. injections of tumor cells and did not develop tumors. The possible value of BCG in human cancer therapy is suggested; BCG injections appear to be a safe form of local therapy for colonic carcinoma.

0295 THE RELEASE OF FOUR MEDIATORS OF IMMEDIATE HYPERSENSITIVITY FROM HUMAN LEUKEMIC BASOPHILS. (Eng.) Lewis, R. A. (Harvard Med. Sch., Boston, Mass.); Goetzl, E. J.; Wasserman, S. I.; Valone, F. H.; Rubin, R. H.; Austen, K. F. *J. Immunol.* 114(1):87-92; 1975.

The availability of a patient with basophilic leukemia manifesting 75-90% mature basophils permitted the use of a cell concentration (3×10^7 leukemic basophils) sufficient to generate and release mediators upon interaction with a calcium ionophore, in quantities adequate for their physicochemical characterization. The mediators were: slow reacting substance of anaphylaxis (SRS-A), which was characterized by purification through silicic acid chromatography and inactivation by arylsulfatase; eosinophil chemotactic factor of anaphylaxis (ECF-A), characterized by gel filtration through Sephadex G-25 and inactivation by subtilisin but not by trypsin; and platelet-activating factor (PAF), characterized by inherent binding to albumin. Both the ECF-A and histamine were present in their preformed state. The histamine concentration (1.3 $\mu g/10^6$ cells), was comparable to that of normal human basophils. Dibutyryl cyclic AMP suppressed release of histamine and the SRS-A, indicating that their availability was under a control similar to that observed with normal cells subjected to immunologic activation. It is concluded that a single cell type can serve as a source of the four recognized mediators of immediate-type hypersensitivity. These results also suggest that the failure of leukemic basophils to respond to direct or reversed anaphylactic challenge resides in the activation phase of the mediator formation and release reaction.

0296 ROSETTE-FORMING ABILITY OF THYMUS-DERIVED LYMPHOCYTES IN HUMORAL AND CELL-MEDIATED IMMUNITY. II. HELPER CELL ACTIVITY. (Eng.) Elliott, B. E. (McGill Cancer Res. Unit, Montreal, Canada); Haskill, J. S. *J. Exp. Med.* 141(3):600-607; 1975.

The helper cell activity in immune and nonimmune C571/6 mouse spleen was measured to determine if these cells form rosettes by binding sheep RBC. Helper cells were assayed by an adoptive transfer system into lethally-irradiated syngeneic recipients. Spleens were assayed for 19S and 7S plaque-forming cells (PFC) eight days later. A velocity sedimentation technique was used to measure rosette-forming ability and size of the helper cells. 19S helper cells in immunized animals were medium lymphocytes, whereas in nonimmune animals they were small lymphocytes. None of the small or medium 19S lymphocytes were rosette-forming. In immune animals, 7S helper cells were non-rosette forming small lymphocytes. It is suggested that the 7S and 19S small and medium lymphocyte helper cells may represent different stages of differentiation. The helper cells described in this work were different from medium lymphocyte effector cells in cell-mediated immunity, which do form rosettes. It remains to be established if these two cell types are at different stages of differentiation or are representatives of different cell lines.

0297 *IN VIVO* AND *IN VITRO* TESTS OF INHIBITORY EFFECT OF PROGESTERONE ON CELL-MEDIATED IMMUNITY IN RATS BEARING A SYNGENEIC UTERINE ADENOCARCINOMA. (Eng.) Sekiya, S. (Chiba Univ. Sch. Med., Japan); Kamiyama, M.; Takamizawa, H. *J. Natl. Cancer Inst.* 54(3):769-771, 1975.

To determine if progesterone may be immunosuppressive, a colony inhibition (CI) assay was used to determine the effects of *in vitro* progesterone and *in vivo* medroxyprogesterone on inhibition or stimulation of uterine adenocarcinoma target cell (HTP/CI) growth. Sensitization against the tumor was produced by s.c. inoculation of about 10^6 viable cells into male rats 10-14 days old. Spleen cell suspensions (10^7 cells/ml) were treated with 0.8, 8, and 16 µg progesterone/ml for two hr in a 5% CO_2 incubator. Spleen cells taken from rats with regressed tumors inhibited colony growth after four weeks of inoculation whereas spleen cells from rats with rapidly growing tumors or metastases increased target cell colony growth. Cell-mediated immunologic activity was not observed with cells taken from tumor-bearing rats two weeks after inoculation. *In vitro* treatment of spleen cells with 8 µg/ml progesterone for two hr produced the greatest suppression of activity of sensitized spleen cells and increased colony growth. The cytocidal effect of sensitized spleen cells from rats with regressed tumors was inhibited when rats received injections of 0.5 mg medroxyprogesterone acetate *in vivo*. The results indicate that the suppressive action of progesterone was due to a specific suppression of immune spleen cells, as the viability of progesterone treated and untreated cells was not different.

0298 TUMOR-SPECIFIC CELL-MEDIATED IMMUNITY IN HOUSEHOLD CONTACTS OF CANCER PATIENTS. (Eng.) Byers, V. S. (Sch. Med., Univ. California, San Francisco); Levin, A. S.; Hackett, A. J.; Fudenberg, H. H. *Scand. J. Clin. Invest.* 34(4): 500-513; 1975.

A short-term chromium-51 release assay was used to test patients with osteogenic sarcoma and related tumors, hypernephroma, and breast carcinoma for tumor-specific cell-mediated immunity against these tumors; household contacts of these patients were

also tested. Tumor-specific immunity was studied primarily against two of the ten cell lines found suitable for the assay procedure: the ALAb breast carcinoma and the Te 85 osteogenic sarcoma. Peripheral blood lymphocytes from each subject were incubated with the target cells, which had previously been incubated with (^{51}Cr) chromium sulfate. The amount of chromium-51 released from the target cells served as an indication of the degree of lymphocyte-mediated cell lysis. The assay was reproducible over many months and correlated well with the clinical course of the patients. It showed that household contacts of patients with osteogenic sarcoma and breast carcinoma had specific immunity against the tumor type with which they had been in contact. In both types of tumors, the range of cytotoxicity values produced by lymphocytes from the household contacts was significantly higher than that of a normal control population. The incidence of immunity was much higher among the household contacts of patients with breast carcinoma than among those of patients with osteogenic sarcoma. Immunity was found with equal frequency in men and women, as well as in genetically related and unrelated household contacts. Immunity against hypernephroma was not demonstrated either in patients with this tumor or in their household contacts. The results suggest that genetic relationship to a cancer patient is not necessary for immunity and that the immunogen involved is transmitted horizontally.

0299 MECHANISM OF THE IMMUNOSUPPRESSIVE EFFECT OF EHRLICH ASCITIC TUMOUR. (Eng.) Hršak, I. (Lab. Exp. Ther., Inst. "Ruder Bošković", Zagreb, Yugoslavia); Marotti, T. *Eur. J. Cancer* 11(3):181-185; 1975.

The mechanism of the immunosuppressive effect of Ehrlich ascitic tumor was investigated in male and female LAF_1 mice (hybrids between C57BL and A strains). Mice were given one i.p. injection of 0.3 ml of the cell-free Ehrlich ascitic fluid (EAF). Seven to 14 days later, the generation of antibody-forming cells in their spleens was suppressed. However, when spleen cells from EAF-treated mice were transferred into normal lethally irradiated recipients, the cells were able to generate normal numbers of antibody-forming cells. Spleen cells or bone marrow and thymus cell mixtures from normal donors were also completely inefficient in producing antibody forming cells when assayed in EAF-treated and irradiated recipients. Incubation of spleen cells, bone marrow cells or thymus cells with EAF prior to transplantation, as well as simultaneous injection of spleen cells together with EAF into irradiated animals, did not inhibit the normal immune performances of these cells. These data suggest that EAF may induce immunosuppression by changing the microenvironment in the spleen, rather than by acting directly on the cells involved in the immune response.

0300 THE EFFECT OF AN IMMUNOSUPPRESSIVE DRUG (AZATHIOPRINE) ON THE LATENT INFECTION OF MARMOSET MONKEYS (*CALLITHRIX JACCHUS*) WITH THE ONCOGENIC *HERPESVIRUS SAIMIRI*. (Eng.) Steinke, H. (Hygiene-Institut der Universität D-3400 Göttingen, Kreuzbergring 57, West Germany); Laufs, R.; Perings, E.; Junge, U. *Med. Microbiol. Immunol. (Berl.)* 161 (3):171-174; 1975.

Herpesvirus saimiri (HVS) causes a latent infection in *Callithrix jacchus* (CJ) marmoset monkeys. To determine whether this resistance to the oncogenic potential of HVS could be due to immune surveillance azathioprine (3 mg/kg) was given daily in food to seven latently infected CJ marmosets. Three of the seven were inoculated with cell-free HVS on day 35 of azathioprine treatment, while four recieved HVS-carrying lymphocytes from an owl monkey on the first day of immunosuppressive treatment. The three monkeys given cell-free HVS died within 114 days after infection, presumably as a result of azathioprine-induced anemia. Gross pathology and histopathology of spleen, liver, and lymph nodes revealed no sign of malignancy. None of the four monkeys challenged with HVS-carrying lymphocytes developed a malignant lymphoma, but all died of anemia within 147 days. These results show that azathioprine does not change latent HVS infection of CJ marmosets into a malignant disease.

0301 THE EFFECT OF B-CELL IMMUNOSUPPRESSION ON AGE-RELATED RESISTANCE OF CHICKENS TO MAREK'S DISEASE. (Eng.) Sharma, J. M. (U. S. Dep. Agric., Agric. Res. Serv., Reg. Poult. Res. Lab., East Lansing, Mich.); Witter, R. L. *Cancer Res.* 35(3):711-717; 1975.

White Leghorn chickens were injected with cyclophosphamide (3 mg/kg/day) for four days following hatching in order to examine the role of bursal dependent functions in the resistance of older chickens to Marek's disease (MD). At eight or nine weeks of age, cyclophosphamide-treated (bursectomized) and control chickens were injected with MD virus. Blood cells obtained from the bursectomized and control animals at this time were able to produce graft *versus* host reactions in allogeneic embryos, indicating that thymus function was intact. B-cell function (measured by the ability to produce antibodies against MD virus, the presence of germinal centers in cecal tonsils, and alterations of lymphoid cell morphology in the bursa of Fabricius) was suppressed or eliminated in cyclophosphamide-treated chicks. No differences between bursectomized and control animals were seen with respect to response to MD. Non-proliferative lesions which lacked plasma cells were seen in peripheral nerve sections obtained from bursectomized chicks. It is concluded that bursa-dependent humoral immunity functions have no effect on the pathogenesis of MD, and that the plasma cell may not be involved in recovery from MD.

0302 NONSPECIFIC AND SPECIFIC IMMUNOSUPPRESSION IN TUMOUR-BEARING MICE BY SOLUBLE IMMUNE COMPLEXES. (Eng.) Gorczynski, R. M. (Dep. Zool., Univ. Coll. London, England); Kilburn, D. G.; Knight, R. A.; Norbury, C.; Parker, D. C.; Smith, J. B. *Nature* 254(5496):141-143; 1975.

Sera and antigen-antibody complexes eluted from the kidneys of BALB/c mice carrying tumors induced by

Moloney sarcoma virus (MSV) were used to study nonspecific and specific suppression of T lymphocyte responses to phytohemagglutinin (PHA), lipopolysaccharide (LPS) or cell surface antigen C-Vgs. Serum taken from mice 14 days after MSV infection significantly inhibited PHA and C-Vgs stimulation of DNA synthesis by T cells, but did not affect B cell response. The serum factors appeared at approximately the same time that the tumors began to regress. Rabbit anti-mouse immunoglobulin (anti-MIg) columns were used to attempt removal of the suppressive activity from the sera of mice 20 days after MSV inoculation, from supernatants of cultures of suppressor cells from MSV progressor mice, and from material eluted from the kidneys 20 days following MSV inoculation. None of the preparations from normal animals suppressed T cell function, whereas all of the preparations from MSV-infected mice caused antigen-specific (C-Vgs) and nonspecific (PHA) suppression. Effluents from anti-MIg columns blocked only the antigen-specific response. The suppression was best seen when column-effluent and column-absorbed materials were recombined. It is suggested that nonspecific T cell blocking is mediated by antigen-antibody complexes, while antigen-specific blocking may be mediated by free antigen or antigen-antibody complexes.

0303 VIRUS EXPRESSION AND IMMUNOPROPHYLAXIS OF A MURINE LYMPHOMA. (Eng.) Sekine, I. (Cent. Health Sci., Univ. California, Los Angeles); Vredevoe, D. L.; Hays, E. F. *J. Natl. Cancer Inst.* 54(3):727-731; 1975.

In order to study the relationship of virion expression to resistance development, CBA mice were injected i.p. with 100 CBA Gross virus-induced lymphoma cells following pretreatment with various tissues expressing or not expressing murine leukemia virus (MuLV). The presence of MuLV was denoted by an *in vitro* quantitative plaque-formation assay. Acceleration of the onset of lymphoma in newborn AKR mice given i.p. injections of the test cell-free filtrates constituted a positve oncogenicity assay (NB+). Injection of 100 CBA lymphoma cells into CBA mice resulted in 100% mortality within 23 days. Filtrates of AKR lymphomas which were XC+ and NB+ protected CBA mice for a minimum of 56 days. AKR normal tissues which were XC+ also protected CBA mice against the CBA lymphoma. Pretreatment with C3H lymphomas offered protection only when they were XC+, NB+ or XC-, NB+. Injections of SJL/J peritoneal exudate cells which were XC+ provided protection against the CB lymphoma, as did SJL/J skin grafts 77 days before challenge. Protection by AKR lymphoma cells occurred only when challenge with CBA lymphoma was made after AKR cell immunization. Macrophage migration of peritoneal cells from CBA mice immunized with AKR lymphomas was inhibited by CBA lymphoma cells *in vitro*. The results indicate that the protective effect offered by immunization is related to the presence of MuLV in the tissues used as antigen. The authors postulate that the protective effect is due to the induction of an early cellular immune response which prevents lymphoma growth.

0304 ANTISERA TO ACUTE LYMPHOBLASTIC LEUKEMIA CELLS. (Eng.) Greaves, M. F. (Imp. Cancer Res. Fund Tumour Immunol. Unit, Univ. Coll., London, England); Brown, G.; Rapson, N. T.; Lister, T. A. *Clin. Immunol. Immunopathol.* 4(1):67-84; 1975.

Antisera raised against acute lymphoblastic leukemia (ALL) cells were tested against lymphoid cells from patients with ALL, other lymphoreticular cancers, and a variety of normal and treated lymphoid cells and related tissues. Three rabbits were given two i.v. injections of 2×10^8 viable ALL lymphoblasts spaced 14 days apart, and were bled on day 21. ALL cells were coated with rabbit antibodies to tonsil lymphocytes by adding 0.5 ml anti-tonsil antiserum to 1 ml ALL cells and rotating for 60 min at 4 C. Two of the three animals were injected with the coated cells. The third rabbit received ALL cells pretreated with normal rabbit serum. Anti-ALL sera were heated at 56 C for 30 min and absorptions were carried out with AB RBC, liver cells, and tonsil cells, using a 5:1 serum/packed cell ratio (60 min, at 4 C). Tests for reactivity of antisera with cells were carried out by indirect immunofluorescence using fluorescein-conjugated goat anti-rabbit immunoglobulin. The two antisera raised against cells coated with anti-tonsil antibody required four tonsil absorptions to reveal discriminatory anti-ALL titers of 16 and 64. In contrast, the antiserum raised with uncoated ALL cells required nine tonsil absorptions to reveal an anti-ALL titer of only 2. Fourteen out of 19 ALL patients at presentation had circulating cells reactive with the antisera. Three of these had a bone marrow diagnosis of ALL with no apparent abnormal cells in the peripheral blood. Of the five unreactive cases, three were the only T cell-like leukemias in the ALL group. Of other types of leukemia and lymphoreticular cancers studied, the only other anti-ALL reactive cells were from two cases of acute undifferentiated leukemia and from four out of five cases of chronic myeloid leukemia in blast crisis. Very weak reactivity occurred in a proportion of cases of acute myeloid leukemia. The reactivity of myeloid leukemia cells could be abolished by absorption with leukemic myeloblasts, leaving ALL reactivity intact. Various control studies suggested that the antigen detected by anti-ALL sera was not a cell cycle, fetal, or cryptic "normal" antigen of lymphocytes. It is concluded that antisera to ALL may define an antigen which is restricted to a large subgroup of ALL cases, and which offers considerable diagnostic and prognostic potential.

0305 EFFECT OF ANTIMYELOMA CELL ANTISERUM ON IMMUNOLOGICAL ENHANCEMENT. (Eng.) Hosokawa, M. (Hokkaido Univ. Sch. Med., Sapporo, Japan); Mihich, E.; Watanabe, T.; Pressman, D. *Cancer Res.* 35(3):591-595; 1975.

The effects of antimyeloma cell antiserum (AMS) on the growth of two immunologically enhanced tumors (Sarcoma 180 and SMT-S) in mice were investigated. Frozen-thawed homogenates of Sarcoma 180 (FTH) and normal rabbit serum were used for immunization. FTH was rejected in 100% of AKR mice after s.c. implan-

tation. Twelve of 35 AKR mice immunized with FTH died bearing large tumors. Tumor rejection by non-immunized mice was not affected by AMS, and neither was it altered in immunized mice if AMS was given before tumor transplantation. AMS treatment decreased tumor rejection in immunized AKR mice if given after tumor transplantation. Normal DBA/2J mice implanted with SMT-S tumors rejected them within 30 days, but five of eight mice died with large tumors when treated with AMS. The results indicate that AMS did not inhibit immunological enhancement of FTH in AKR mice, and that AMS reduced the incidence of rejection in both immunologically enhanced tumors studied.

0306 STRUCTURAL, FUNCTIONAL, AND IDIOTYPIC CHARACTERISTICS OF A PHOSPHORYLCHOLINE-BINDING IgA MYELOMA PROTEIN OF C57BL/ka ALLOTYPE. (Eng.) Claflin, J. L. (Washington Univ. Sch. Med., St. Louis, Mo.); Rudikoff, S.; Potter, M.; Davie, J. M. *J. Exp. Med.* 141(3):608-619; 1975.

Plasmacytoma CBPC 2 was induced in CB-20 mice (a strain congenic to BALB/c, but differing by carrying the A^{15} allotypic determinant found in C57BL/ka mice) to characterize an IgA protein produced by the myeloma which binds phosphorylcholine (PC). The IgA PC-binding protein has different IgC_H allotypic determinants from those of BALB/c mice. Sequence and isoelectric point analysis revealed the light chain of the CBPC 2 protein to be the same as the light chain of T15, a protein which binds PC and is produced by myelomas in BALB/c mice. The heavy chains of the T15 and CBPC myeloma proteins differ by two amino acids at positions 14 and 16. Both proteins were found to have similar specificities for acetylcholine, choline, glycerophosphorylcholine and PC. A binding site associated idiotypic determinant present on the T15 protein was also found on the CBPC 2 protein. The non-binding site idiotypic determinant was not present in CBPC 2 protein. This is similar to findings in normal and induced C57BL/6 anti-PC antibody. It is concluded that the structural differences in these proteins relate to allotypic characteristics in the C_H region or to a difference in a variable region outside the combining site. Thus, the combining site is similar in structure in two strains of mice that differ considerably genetically.

0307 PARTIAL AMINO ACID SEQUENCE OF THE PRECURSOR OF IMMUNOGLOBULIN LIGHT CHAIN PROGRAMMED BY MESSENGER RNA *IN VITRO*. (Eng.) Schechter, I. (Weizmann Inst. Sci., Rehovot, Israel); McKean, D. J.; Guyer, R.; Terry, W. *Science* 188(4184):160-162; 1975.

Messenger RNA (mRNA) isolated from polysomes obtained from MOPC-321 mouse myeloma was specifically precipitated with antibodies to the immunoglobulin light (L) chain and incubated in a cell-free protein synthesis system to characterize the L chain protein precursors coded by the L-chain mRNA. The proteins produced were resolved using sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis. ^{3}H-leucine was used in the incubation in order to label the proteins produced. Five proteins were separated which had 20 amino acid residues preceding the amino terminus of the complete protein, and which were labeled at the same points. The labeling pattern differed from that of the mature protein. There was an abundance of leucine residues in the extra piece (20 amino acid residues) coupled to the NH_2-terminus of the mature protein, indicating that it is hydrophobic. The results indicate that there is one major point of initiation of MOPC-321 L-chain mRNA translation. It is suggested that the L-chain precursor studied may have an extra piece at the carboxyl terminus as well.

0308 LOCAL MONOCLONAL IMMUNOGLOBULIN PRODUCTION IN CANCER PATIENT. (Eng.) Górny, M. K. (Med. Acad., Poznán, Poland); Zeromski, J. *Experientia* 31(2):238-239; 1975.

The case report of a 68-yr-old man with rectal adenocarcinoma whose plasma cells produced monoclonal immunoglobulin G (IgG) is described. Plasma cells were identified in the tumor itself, surrounding mucosa and regional lymph nodes by means of a battery of monospecific immunofluorescene reagents. The relative quantity of plasma cells was determined by a cellular density index (CDI). The CDI for the regional lymph nodes was very high for IgG-producing cells which constituted 90% of the total plasma cell number. The remaining 10% consisted of IgA, IgM and IgE-producing cells. The patients displayed hypoproteinemia (total protein 3.4 g/100 ml). Quantitative serum immunoglobulin determinations revealed a decrease of IgG and IgA and an increase of IgM. Both paper electrophoresis and immunoelectrophoresis did not show monoclonal protein in serum. These data show that regional lymph nodes in cancer may be a site of monoclonal Ig synthesis and that this phenomenon may not be detectable in the patient's serum.

0309 CELL CYCLE-ASSOCIATED CHANGES IN RECEPTORS FOR IgE DURING GROWTH AND DIFFERENTIATION OF A RAT BASOPHILIC LEUKEMIA CELL LINE. (Eng.) Isersky, C. (Natl. Inst. Arthritis, Metab. Dig. Dis., Bethesda, Md.); Metzger, H.; Buell, D. N. *J. Exp. Med.* 141(5):1147-1162; 1975.

Changes in the IgE binding activity of rat basophilic leukemia cells (RBL-1) during culture growth and transit through the cell cycle were investigated. Three growth patterns were examined: (1) cells from a stationary phase culture were resuspended in fresh medium and allowed to reach high density and remain in stationary phase for up to 72 hr, (2) cultures were maintained in exponential growth by daily dilutions with fresh medium at a ratio of 1:2 or 1:3, and (3) synchronized cultures were derived from exponential cultures by using a double thymidine block procedure. A variable number (2.4 to 14 x 10^5) of cells were incubated with 0.145 μg [^{125}I]IgE. There was an inverse relationship between growth rate and expression of receptor activity for IgE. After prolonged exponential growth, the number of receptors per cell stabliized at 4 to 6 x 10^5. Cells in stationary cultures, which were arrested in the G_1

phase of the cell cycle, continued to accumulate up to 0.9 to 1.7 x 10^6 receptors per cell with no increase in volume. Upon resuspension in fresh medium at low density (3 to 5 x 10^5 cells/ml), these cells lost up to 70% of the receptor activity within four hr. Assessment of cultures synchronized by double thymidine block and cells fractionated by centrifugation of a Ficoll gradient indicated that the RBL-1 cells acquired receptors in the G_1 phase of the cell cycle. No accumulation of active receptors occurred during the S and G_2 phases, although the average cell volume increased. Cell division resulted in a drop in number of receptors per cell while the number of cell-bound receptors in the culture remained unchanged. This indicates that during mitosis receptors were simply distributed in daughter cells. The results indicate that the number of detectable IgE receptors on the cultured rat basophils varies significantly during the cell cycle and that the average number of receptors expressed by cells in a given culture could be controlled by varying the growth conditions.

0310 INTERACTION OF IgE WITH RAT BASOPHILIC LEUKEMIA CELLS. III. RELEASE OF INTACT RECEPTORS ON CELL-FREE PARTICLES. (Eng.) Carson, D. A. (Natl. Inst. Arthritis, Metab. Dig. Dis., Bethesda, Md.); Kulczycki, A., Jr.; Metzger, H. *J. Immunol.* 114(1):158-160; 1975.

Experiments have been performed on rat basophilic leukemia cells which indicate that as the cells lose viability they release particulate material which contains the receptors for immunoglobulin E (IgE) found on the living cells. Up to 50-80% of the receptors appeared to be recovered in the particulate fraction. Semi-quantitative studies indicated that the binding activity is equivalent in strength and in specificity to the native receptors. Dissociation studies showed that the previously determined K-1 at 4 C may have been falsely high because of the release of receptors during the experiment and that the true value is likely to be less than 5 x 10^6/sec. It is suggested that the high yields and stability of the particulate fraction may make it a useful preparation from which to purify further the receptor for IgE.

0311 THYMUS REACTIVE IgM AUTOANTIBODIES IN NORMAL MOUSE SERA. (Eng.) Martin, W. J. (Zool. Dep., Univ. Coll., London, England); Martin, S. E. *Nature* 254(5502):716-718; 1975.

The occurrence of IgM antibodies reactive with autologous thymus cells in normal mouse sera is reported and demonstrated in the autoantibody nature of certain naturally occurring antibodies (NOA). Sera of C3H/HeIerf mice (C3H) were tested in the trypan blue dye exclusion cytotoxicity assay for complement-dependent cytotoxic activity against syngeneic thymus and spleen cells. In nine out of ten experiments, serum of normal C3H mice caused significant lysis of syngeneic thymus cells. In 12 experiments, the percentage lysis of syngeneic spleen cells by normal C3H serum varied from 5.4% to 7.3%. The anti-thymus cytotoxic activity of normal C3H serum was rabbit complement (RC') dependent. The anti-thymus cytotoxic activity of normal C3H serum was attributed to naturally occurring IgM antibodies since the cytotoxicity could be absorbed from mouse serum with anti-mouse immunoglobulin antibody-bound sepharose beads, and by a monospecific preparation of goat anti-mouse IgM antibody. Sera of all strains tested were cytotoxic for autologous thymus cells when undiluted normal sera of various strains were tested for RC' dependent cytotoxicity against autologous thymus cells. Thymus cells of hydrocortisone-treated normal mice were susceptible to lysis by NOA. Thymus cells obtained from neonatal mice were lysed but only by undiluted normal serum. The finding of thymus reactive antibodies in sera of congenitally athymic mice indicates that the thymus is not required as the antigenic source for the production of such antibodies and that the antibody response in normal mice is probably thymus independent.

0312 EB-VIRUS-SPECIFIC IgM AND IgG ANTIBODIES IN FIRST-DEGREE RELATIVES OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA. (Eng.) Zorbala-Mallios, H. (King's Coll. Hosp. Med. Sch., London, England); Sutton, R. N. P.; Emond, R. T. D. *Arch. Dis. Child.* 50(2):137-141; 1975.

Evidence of Epstein-Barr (EB) virus infection was looked for in mothers and sibs of children with acute lymphoblastic leukemia (ALL), in patients with infectious mononucleosis (IM), and in healthy controls. IgM and IgG fractions were chromatographically separated from the serum samples taken from these subjects and were tested by indirect immunofluorescence for antibodies to EB virus capsid antigen (VCA) and EB complement fixing (CF) antigen. IgM VCA antibody was detected in 14/16 patients with IM, and IgG VCA antibody was found in 12. IgM CF antibody was not detected in any of the IM specimens, but IgG CF antibody was present in 15/16. IgG CF antibodies were found in 2/4 control patients with other infectious diseases, none of whom had IgG antibodies. Twelve of the 16 mothers with ALL children had EB-specific IgM VCA antibodies, and 15 had IgG VCA antibodies; all had detectable IgG CF antibody while none had IgM CF antibody. No IgM antibodies were found in six mothers of healthy children, although 1/2 mothers of hospitalized non-ALL children had such antibodies; half of the control mothers had IgG CF antibodies. Of sibs of the ALL children, three had IgM VCA antibodies, all had IgG VCA antibodies, and one had IgG CF antibodies. The results support the view that EB virus infection is associated in some way with ALL.

0313 CLINICAL EVALUATION OF PATIENTS WITH INFECTIOUS MONONUCLEOSIS AND DEVELOPMENT OF ANTIBODIES TO THE R COMPONENT OF THE EPSTEIN-BARR VIRUS-INDUCED EARLY ANTIGEN COMPLEX. (Eng.) Horwitz, C. A. (Univ. Minnesota Sch. Med., Minneapolis); Henle, W.; Henle, G.; Schmitz, H. *Am. J. Med.* 58(3):330-338; 1975.

Fourteen patients with infectious mononucleosis who demonstrated antibodies to the restricted (R) component of the early antigen (EA) complex only, or

who evidenced anti-R several weeks or months following the usual initial anti-diffuse (anti-D) response were examined. In 6/7 patients in whom anti-EA responses were solely anti-R, primary Epstein-Barr virus (EBV) infections were demonstrated. In two of these patients, anti-R levels were undetectable 3-9 months later; in four of them, anti-R was present 9-23 months later. In the seven patients that responded with the characteristic initial anti-D response, anti-R could only be detected when anti-D titers fell. In four of the patients, anti-R could be demonstrated 6-39 months later. Unusual features of the clinical course were seen in three of the seven patients with no anti-D response, and in five of the seven with initial anti-D response. The patients with a protracted course of disease generally were those who retained anti-R activity for the longest time. It was concluded that some patients with infectious mononucleosis may respond with a rise in anti-R rather than anti-D, or may reveal anti-R when anti-D titers fall. The protracted high levels of anti-R and clinical symptoms may represent a greater than usual EBV persistence.

0314 INTERFERON PRODUCTION IN HODGKIN'S DISEASE. (Eng.) Chisholm, M. (Professorial Medical Unit, Univ. Southampton, Southampton, England); Cartwright, T. *Clin. Exp. Immunol.* 20(3):419-425; 1975.

The ability of WBC from patients (21 untreated and 21 treated with radiotherapy or chemotherapy) with Hodgkin's disease to produce interferon in response to a viral challenge was investigated. WBC suspensions from these patients and from 33 controls were incubated with Sendai virus (100 hemagglutinating U/ml) at 37 C for 18 hr. Interferon production was assayed by adding serial dilutions of interferon-containing solutions to plated green monkey cells prior to exposure to Sindbis virus (100 TCD_{50} U/ml); the reciprocal of the interferon solution protecting 50% of the cells against cytopathological effects was taken to be the interferon titer of that solution. The mean titer in untreated patients (233.6 U) did not differ from that of the controls (210.4 U), whereas that of the the treated patients was significantly depressed (10.5 U). Lymphocyte transformation by phytohemagglutinin (10 µl/ml cell suspension) was simultaneously assayed in 19 controls and 21 patients with Hodgkin's disease. Although lymphocyte transformation did not correlate directly with the interferon titer, it was significantly depressed in Hodgkin's patients compared with controls, and the depression was most marked in the treated patients. The total and differential WBC count and the stage of disease did not appear to relate to the interferon titer. Titers were normal in most of 11 patients with untreated stage IV disease and in all but one of 15 patients with stages I, II, and III disease. This patient was receiving radiotherapy and prednisone at the time of the study. These results do not support the concept that defective interferon synthesis is a factor in the pathogenesis of Hodgkin's disease. However, the findings may explain the previously reported increase in herpes zoster varicella infection in treated patients with Hodgkin's disease.

0315 IMMUNE SPECIFIC PRODUCTION OF INTERFERON BY HUMAN T CELLS IN COMBINED MACROPHAGE-LYMPHOCYTE CULTURES IN RESPONSE TO HERPES SIMPLEX ANTIGEN. (Eng.) Valle, M. J. (Stanford Univ. Sch. Med., Calif.); Bobrove, A. M.; Strober, S.; Merigan, T. C. *J. Immunol.* 114(1):435-441; 1975.

The immune specific production of interferon by human T and B cells in combined macrophage-lymphocyte cultures in response to stimulation by herpes simplex (HSV) antigen was studied. Human peripheral blood lymphocytes, highly enriched for T cells, were obtained by passing gravity-sedimented leukocytes through nylon wool columns. The eluted cells were then cultured with autologous macrophages and the mixed cultures stimulated by 0.04 ml of HSV antigen; 0.04 ml of diluted phytohemagglutinin (PHA) was added to some of the cultures. The transformation response of the stimulated cultures was assayed by measuring the incorporation of ^{3}H-thymidine and the interferon activity in the culture supernatants was assayed by their ability to inhibit the plaque formation of vesicular stomatitis virus in human foreskin fibroblast cultures. The interferon produced by the combined macrophage-lymphocyte cultures was dependent upon the presence of T cells; elimination of these cells by treatment with an anti-T serum plus complement greatly diminished the amount of interferon produced. The memory for the immune-specific release of interferon also appeared to be carried by the T lymphocytes rather than the glass-adherent macrophages. Both PHA and HSV antigen stimulated the production of interferon by the mixed cultures. The results suggest that the immune-specific interferon originated from the T lymphocytes alone and that the macrophages simply provided an antigen trapping and delivery function for the T cells.

0316 CARCINOEMBRYONIC ANTIGEN IN PATIENTS WITH GYNECOLOGIC MALIGNANCY. (Eng.) van Nagell, J. R., Jr. (Univ. Kentucky Med. Cent., Lexington); Meeker, W. R.; Parker, J. C., Jr.; Harralson, J. D. *Cancer* 35(5):1372-1376; 1975.

Carcinoembryonic antigen (CEA) levels were measured in sera obtained from 100 patients with gynecologic malignancies, 95 patients with benign gynecologic disease, and 176 healthy volunteers to determine the usefulness of CEA assay in the diagnosis of malignancy and to discover any relationship of CEA levels to cell type and tumor differentiation. Elevated CEA levels (above 2.5 ng/ml) were observed in 81% of the patients with malignancies, in 18% of the patients with benign disease, and in 11% of the healthy volunteers. Nine of 40 patients with carcinoma of the cervix had the highest CEA levels, while patients with ovarian and endometrial carcinoma had the most consistently elevated CEA levels (17 of 18 were over 2.5 ng/ml). The incidence of elevated CEA levels was highest in adenocarcinoma patients. Elevated CEA levels were seen most often in patients with benign uterine pathology rather than in ovarian or tubal problems. Cancerous tissues from 81 of 100 patients with malignancies were obtained and examined histologically. Vascular in-

vasion was the only characteristic which correlated with elevated CEA titers. Fourteen of 16 patients with elevated CEA titers and whose tumors were completely removed surgically had normal titers eight weeks following removal. The results indicate that elevated CEA levels correlate well with malignancy and thus may be useful in the diagnosis and management of gynecologic carcinomas.

0317 DISTINCTION BETWEEN TUMOR-SPECIFIC TRANSPLANTATION ANTIGEN AND VIRION ANTIGENS IN SOLUBILIZED PRODUCTS FROM MEMBRANES OF VIRUS-INDUCED LEUKEMIC CELLS. (Eng.) Chang, K. S. S. (Natl. Cancer Inst., Bethesda, Md.); Law, L. W.; Appella, E. *Int. J. Cancer* 15(3):483-492; 1975.

A membrane antigen from RBL-5 leukemic cells that was solubilized and partially purified is characterized as a new surface component. Inhibition of the growth of FBL-3 cells was observed using adoptively transferred sensitized lymphoid cells (Winn assay). There was a significant reduction in the number of takes of a 5 x 10^2 cell challenge with FBL-3 leukemic cells in B1/6 mice given a single immunization with 30 μg or 100 μg of the F2 fraction of RBL-5 antigen preparation. In similar assays the crude soluble (CS) material obtained from limited papain digestion of the membranes of FBL-3 cells also contained a striking immunogenic potential or i.p. challenge with 1 x 10^3 FBL-3 cells. The immunogenicity of 150 μg was essentially that obtained with 1 x 10^6 viable FBL-3 cells inoculated s.c. The absence of infectious type C viruses in the membrane preparations of RBL-5 was confirmed by the inability of these preparations to induce production in ME cells of progeny viruses detectable by measurement of virion associated activity of reverse transcriptase using oligo(dT) poly(rA) as a primer template. Virus neutralizing antibody was not detected, under stringent assay conditions, in the syngeneic anti-RBL-5 crude membrane (anti-CM) sera. Antigen preparation CM, CS and the chromatographed fractions F1, F2 and F3 were assayed in a complement fixation test against broad-reacting antisera capable of detecting virus envelope antigen and *gs* antigen and against syngeneic antisera. Although the antigen preparations were positive for virion antigens, CS and F2 contained an antigen that reacted only with syngeneic antiserum. These same fractions were those reactive as immunogens. The data are in accord with the concept that trace or moderate amounts of virion antigens may be present in all preparations of solubilized RBL-5 membrane antigens, but that the nonvirion antigen, presumably tumor cell membrane-associated antigen, may be partially purified and concentrated in the F2 fraction.

0318 IDENTIFICATION OF A COLON-SPECIFIC ANTIGEN (CSA) IN NORMAL AND NEOPLASTIC TISSUES. (Eng.) Goldenberg, D. M. (Univ. Kentucky Med. Cent., Lexington); Pegram, C. A.; Vazquez, J. J. *J. Immunol.* 114(3):1008-1013; 1975.

An antigen produced by GW-39 (a signet-ring cell carcinoma) tumors grown in hamsters was isolated and partially characterized. This colon-specific antigen (CSA) was extracted from GW-39 tumors by saline, perchloric acid or phenol and was found to be identical immunologically to antigen extracted from normal hamster and human colon. Immunodiffusion and immunofluorescence studies revealed the CSA to be distinct from CEA, CCA-II, CCA-III (other antigens associated with colonic tumors) and the major blood group-specific antigens. Hamsters immunized with CSA 48 days prior to tumor implantation rejected the tumors and had high titers of anti-CSA antibodies. Antisera from immunized hamsters were found to cross-react with an antigen present in RPMI 2650 (an anaplastic squamous cell carcinoma) tumor cells. Gel filtration of CSA from GW-39 tumor extracts suggested that CSA has a molecular weight of 30,000 to 50,000 daltons. Its solubility properties suggest it is a glycoprotein. It is suggested that CSA analysis could be important in diagnosing ulcerative colitis and mucin-producing adenocarcinoma of the colon.

0319 ALPHA-FETO PROTEIN IN LUNG CANCER METASTATIC TO THE LIVER. (Eng.) Tsung, S. H. (Lab. Serv., Veterans Adm. Cent., Wood, Wis.). *Arch. Pathol.* 99(5):267-269; 1975.

A case study is reported involving a 42-yr-old man with bronchogenic carcinoma and elevated alkaline phosphotase levels of 175 IU/liter. Three months after chest irradiation the patient was readmitted for weakness, emaciation and general deterioration. Laboratory studies revealed the following: hematocrit, 20%; hemoglobin, 6.5 g/100 ml; serum bilirubin, 4.5 mg/100 ml; serum albumin, 3.0 g/100 ml; serum calcium, 8.2 mg/100 ml; serum alkaline phosphatase, 1,600 IU/liter; and serum glutamic oxaloacetic transaminase, 300 IU/liter. Two α-feto protein determinations by counter electrophoresis were positive. The patient expired two months later. An indirect immunofluorescent study was made to localize the site of α-feto protein synthesis. The specificity of the immunofluorescence was evaluated by using three controls: (1) a hepatoma positive for α-feto protein as a positive control, (2) normal liver as negative control and (3) inhibition of the α-feto protein. Negative immunofluorescence was found in tumor cells of lung and liver but positive immunofluorescence was found in normal liver cells adjacent to tumor cells. It is postulated that liver cells around metastic foci undergo regeneration and produce α-feto protein.

0320 THE ANTIGENICITY OF CARCINOEMBRYONIC ANTIGEN IN MAN. (Eng.) MacSween, J. M. (Camp Hill Hosp., Halifax, Canada). *Int. J. Cancer* 15(2):243-252; 1975.

The binding of globulins to ^{125}I-carcinoembryonic antigen (CEA) was measured by a previously-reported dilution method and by radioimmunoelectrophoresis in sera obtained from 26 blood donors and 24 cancer patients and in 27 supernatants of tumor (colon, stomach, lung and breast) cell suspensions. Binding of more than 10% ^{125}I-CEA was found in 13/26 blood donors, 10/24 cancer patients and 7/26 tumor cell

supernatants. All five supernatants from lung tumor showed binding which was significantly greater than binding in supernatants from the other tumors studied. Addition of 6 ng unlabeled CEA inhibited binding by ^{125}I-CEA in one cancer serum sample. Binding was reduced by adsorption with blood group A RBC in two cancer cell supernatants and 1/5 sera. Binding appeared to be due to IgM in 10/14 sera tested by radioimmunoelectrophoresis. Serum obtained from a patient with cancer of the stomach was compared to anti-CEA for binding activity. Fifty-three percent of anti-CEA could be recovered from the antigen-antibody complex following addition of an equal volume of 60% saturated ammonium sulfate. Binding was not increased in dialyzed precipitates, but increased binding was seen with precipitates of heterologous anti-CEA and CEA. The results indicate that human IgM can bind to CEA and that antibodies to blood group A may be involved, although the binding may be due to a cross-reactivity by antibodies to antigens of similar form, such as blood group A.

0321 FELINE ONCORNAVIRUS-ASSOCIATED CELL MEMBRANE ANTIGEN. III. ANTIBODY TITERS IN CATS FROM LEUKEMIA CLUSTER HOUSEHOLDS. (Eng.) Essex, M. (Harvard Univ. Sch. Public Health, Boston, Mass.); Jakowski, R. M.; Hardy, W. D., Jr.; Cotter, S. M.; Hess, P.; Sliski, A. *J. Natl. Cancer Inst.* 54(3): 637-641; 1975.

Feline oncornavirus-associated cell membrane antigen (FOCMA) antibody and feline leukemia virus (FeLV) group specific antigen (gsa) were measured in 182 healthy cats from 17 households where at least one other virus positive cat was known to reside in order to study horizontal transmission under natural exposure conditions. About 92% of the cats tested were positive for FeLV gsa or FOCMA antibody. The mean antibody titer of the 182 cats was more than four times higher than that of healthy cats with no known FeLV exposure. FOCMA antibody was detectable in 79.6% of the gsa-positive cats and in 83.2% of the gsa-negative cats but the mean titer was two times higher in the latter group. No significant sex or breed differences were noted. The mean antibody titer was higher in cats five yr or older, although slightly fewer animals in this group were gsa-positive. The results indicate that horizontal transmission of FeLv occurs in natural environments and that most cats exposed to FeLV show an immune response to it. The authors suggest that the horizontal transmission of FeLV may be responsible for increased incidences of leukemia in cluster households.

0322 TUMOUR-ASSOCIATED TRANSPLANTATION ANTIGENS OF CHEMICALLY INDUCED SARCOMATA CROSS REACTING WITH ALLOGENEIC HISTOCOMPATIBILITY ANTIGENS. (Eng.) Invernizzi, G. (Division of Experimental Oncology A, Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milano, Italy); Parmiani, G. *Nature* 254(5502):713-714; 1975.

Protection against syngeneic tumors by immunization with normal allogeneic tissues was investigated in two BALB/c fibrosarcomas, ST-5 and B-2. Groups of BALB/c mice were transplanted with skin or with kidney plus liver from syngeneic, C57BL/6J or C3Hf, mice with skin of W/Fu rat, or were injected i.p. with sheep RBC. Ten days later all mice received 400 rad (whole body) and were challenged on the following day with sarcoma ST-5 cells. A significant protection against sarcoma ST-5 (as assessed by X^2 test) was elicited by C57BL/6J skin or liver plus kidney grafts when compared with syngeneic skin graft. C3Hf skin graft was highly effective from day 8 to 32 and a still stronger protection was achieved by C3Hf liver plus kidney graft from day 8 to 36. No resistance could be induced by rat skin graft or by injection of sheep RBC. No effect was induced by C57BL/6J or C3Hf liver plus kidney graft against the challenge of sarcoma B-2. Absorption with ST-5 cells significantly removed the cytotoxic activity of a BALB/c anti-C57BL/6J serum compared with absorption with BALB/c lymphocytes or with sarcoma B-2. In contrast, several attempts to absorb the cytotoxic activity of a BALB/c anti-C3Hf serum with either tumor failed. Growth excision of ST-5 in $CB6F_1$ mice elicited no immunity against a subsequent challenge of the related cells, whereas only a slight reduction in protection was observed in $C3CF_1$ compared with BALB/c mice. It is concluded that the origin of tumor-associated transplantation antigens (TATA) of sarcoma ST-5 is the result of carcinogen-induced random mutations at the level of histocompatibility genes, either of the H-2 system or of the minor loci. Such a possibility is supported by the genetic linkage which seems to occur between TATA and the H-2 system.

0323 TUMOR-SPECIFIC IMMUNITY TO CHEMICALLY INDUCED TUMORS. EVIDENCE FOR IMMUNOLOGIC SPECIFICITY AND SHARED ANTIGENICITY IN LYMPHOCYTE RESPONSES TO SOLUBLE TUMOR ANTIGENS. (Eng.) Forbes, J. T. (Univ. Florida Coll. Med., Gainesville); Nakao, Y.; Smith, R. T. *J. Exp. Med.* 141(5):1181-1200; 1975.

The apparent paradox that methylcholanthrene-induced tumors of mice evoke tumor transplantation immunity but reveal almost complete cross-reacting antigenicity in tests of lymphocyte behavior *in vitro* was investigated. Tumor membranes from female C57BL/6, 10, C57BL/10xBR and C57BL/10xD2 mice were solubilized in 3 M KCl and were employed both as the stimulating antigen source in a new *in vitro* proliferation assay of lymphocyte recognition, and as immunogens *in vivo*. The kinetics of the assay resembled those of *in vitro* tests of mitogen or specific antigen stimulation in other systems. Lymphoid cell proliferation was assessed in peripheral blood leukocytes, lymph nodes, and spleen over the course of tumor bearing, and in animals immunized by tumor amputation or with the solubilized antigens. The pattern of spread of reactivity was from regional lymph nodes to spleen, peripheral blood, and nonregional nodes in each circumstance. An unexplained low antigen dose inhibitory phenomenon was encountered in spontaneously proliferating cell subpopulations taken from some tumor-bearing animals. *In vitro* responses to some but not all solubilized antigens made from multiple syngeneic tumors were detected in each circumstance. The soluble antigens also induced shared resistance

to some tumors. The patterns of spread of responsiveness to syngeneic tumor antigens, the time course, and relative intensity were most compatible with independent clonal responses to multiple tumor-borne antigens, some but not all of which are shared in any family of syngeneic tumors. The results suggest the probable existence of multiple antigenic components on such tumor cells and that some but not all of these components are shared with syngeneic tumors.

0324 TUMOR-SPECIFIC AND FORSSMAN ANTIGENS OF GUINEA-PIG HEPATOMA CELLS: COMPARISON OF TUMOR CELLS GROWN *IN VIVO* AND *IN VITRO*. (Eng.) Evans, C. H. (Natl. Cancer Inst., Bethesda, Md.); Ohanian, S. H.; Cooney, A. M. *Int. J. Cancer* 15(3): 512-521; 1975.

Cloned tumor cell lines derived from two antigenically distinct ascites variants of diethylnitrosamine-induced guinea-pig hepatomas (designated line 1 and line 10) were compared to ascites tumor cells grown *in vivo* in terms of tumor-specific and Forssman surface antigens. Cell surface antigens on the ascites and tumor cells grown *in vitro* were analyzed with immunofluorescence, C1 fixation and transfer, and antibody-complement-mediated cytotoxicity tests. Tumor-specific and Forssman antigens continued to be expressed during 3-6 months of *in vitro* cultivation. Differences between ascites and cultured cells were noted in the degree of antigen expression and sensitivity to antibody-complement-mediated cytotoxicity. Cells grown *in vitro* exhibited a greater number of Forssman and tumor-specific antigen sites than cells grown *in vivo* as determined by the quantitative C1 fixation and transfer test. Immunofluorescent staining indicated that some cloned lines were considerably more homogeneous in terms of antigen expression that were the cultured, noncloned parent cells or ascites-grown cells. Cloned lines were frequently more sensitive to the cytotoxic action of antibody and complement than were the cultured, noncloned parent tumor cells. Sensitivity to cytotoxicity did not necessarily correlate, however, with the degree of antigen expression. The results suggest that: (1) expression of Forssman and tumor-specific antigens does not diminish on cells cultivated *in vitro;* and (2) ascites hepatoma cells *in vivo* are a heterogeneous population of cells differing in their degree of antigen expression and sensitivity to antibody-complement-mediated cytotoxicity.

0325 ISOLATION OF A POLYPEPTIDE THAT HAS LYMPHOCYTE-DIFFERENTIATING PROPERTIES AND IS PROBABLY REPRESENTED UNIVERSALLY IN LIVING CELLS. (Eng.) Goldstein, G. (New York Univ. Sch. Med., N. Y.); Scheid, M.; Hammerling, U.; Boyse, E. A.; Schlesinger, D. H.; Niall, H. D. *Proc. Natl. Acad. Sci. USA* 72(1):11-15; 1975.

The isolation and purification of a polypeptide which induces the differentiation of pro-thymocytes and of B lymphocytes are described. This polypeptide, named ubiquitous immunopoietic polypeptide (UBIP), was isolated by polyacrylamide disc electrophoresis from bovine thymus. The molecular weight of UBIP was estimated to be 8500 on the basis of molecular exclusion chromatography, amino-acid composition, and amino-acid sequence. It induced, *in vitro*, the differentiation of pro-thymocytes and of both T- and B-cells. In contrast, thymopoietin induced pro-thymocyte differentiation but had no effect on pro-B cells; propranolol also blocked both T-cell and B-cell differentiation by UBIP but had no effect on the induction of T-cell differentiation by thymopoietin. A highly specific radioimmunoassay for UBIP was used to show the presence of this polypeptide in the tissues of (*nu/nu*) mice, which have no thymus, mammalian tumor cell lines, fibroblasts, various cells maintained in culture, and avian embryos and fibroblasts in culture; UBIP was also found in fish, higher plants, yeasts, bacteria and in celery. There was a remarkable conservation of amino-acid sequence in the UBIP obtained from these sources, six of the first eight residues being identical in bovine and celery UBIP. Although UBIP is a potent inducer of immunocyte differentiation *in vitro*, it is unlikely to have such a function *in vivo*. It may well be a universal constituent of living cells and its as yet unknown function is probably an integral feature of living cells.

0326 MALIGNANT LYMPHOMAS OF FOLLICULAR CENTER CELL ORIGIN IN MAN. I. IMMUNOLOGIC STUDIES. (Eng.) Leech, J. H. (Vanderbilt Univ. Sch. Med., Nashville, Tenn.); Glick, A. D.; Waldron, J. A.; Flexner, J. M.; Horn, R. G.; Collins, R. D. *J. Natl. Cancer Inst.* 54(1):11-21; 1975.

Immunologic studies were carried out on 26 patients with non-Hodgkin's lymphomas with histologic features indicating a follicular center cell (FCC) origin. Lymphoid cells from 25 lymph node samples, one spleen sample, three bone marrow samples, and two peripheral blood samples were analyzed by direct immunofluorescence, and their capacity to produce surface Ig and to form rosettes with sheep erythrocytes (E) was determined. The results were compared with those obtained from benign, reactive lymphoid tissue from 14 patients. Of the 26 patients with FCC lymphomas, 22 had 40% or more Ig-bearing cells, and all patients with FCC lymphoma tissues had 25% or less E rosette-forming cells. Cells from most FCC lymphomas of the cleaved type had surface IgG and those from several FCC lymphomas had both IgM and IgD. Cells from lymphomas of the noncleaved cell type had surface IgG or IgA. Light-chain analysis showed that the FCC lymphoma cells bore a predominant light-chain type, which indicated their monoclonal nature. Neoplastic cells from several FCC lymphomas synthesized the surface Ig which they bore. The reactive tissues generally contained fewer Ig-bearing and more E rosette-forming cells than the FCC lymphomas, and the Ig-bearing cells, with one exception, had a polyclonal distribution. Correlation of the histologic and immunologic observations indicated that most of the lymphomas identified as FCC in origin by light microscopy were of B cell origin as shown by immunologic techniques; the data also indicate that FCC lymphomas are the most common type of non-Hodgkin's lymphoma.

0327 INHIBITION BY COLCHICINE OF THE MITOGENIC STIMULATION OF LYMPHOCYTES PRIOR TO THE S PHASE. (Eng.) Wang, J. L. (Rockefeller Univ., New York, N.Y. 10021); Gunther, G. R.; Edelman, G. M. *J. Cell Biol.* 66(1):128-144; 1975.

The effects of colchicine on the stimulation of lymphocytes by concanavalin A (Con A) were investigated. At 10^{-6} M, colchicine inhibited the response of human, murine, and rabbit lymphocytes to Con A (20 μg/ml) by 70-90%, as measured by $[^3H]$-thymidine incorporation 48-50 hr after the start of cultures. DNA synthesis in cultures without colchicine began about 30 hr after initial Con A binding, whereas cultures containing colchicine showed only low levels of DNA synthesis as late as 51 hr. These data suggest that colchicine inhibits mitogenic stimulation of lymphocytes by blocking an event occurring as early as the first wave of DNA synthesis in the sequence of events following lectin addition. The effect of colchicine on Con A stimulation of lymphocytes was also manifested by a decrease in the percentage of lymphocytes transformed into blast cells. Differences in DNA synthesis levels and blast transformation between lectin-stimulated cultures with and without colchicine were apparent as early as 36 hr. Therefore, the onset of division in Con A-stimulated cultures occurs too late to support the contention that colchicine acts by metaphase arrest. In addition, colchicine caused no appreciable decrease in viabilities of unstimulated lymphocytes or blast cells. The inhibition of $[^3H]$-thymidine incorporation was not due to blockage of thymidine transport or inhibition of DNA synthesis, because addition of colchicine had an effect on cells in the S phase of the cell cycle. At 10^{-6} M, vinblastine and vincristine also inhibited the response of both human and murine lymphocytes to Con A. Lumicolchicine, a photoinactivated colchicine derivative, had no appreciable effect on blast transformation or DNA synthesis in Con A-stimulated lymphocytes. The findings support the hypothesis that cytoplasmic microtubular function plays a role in the commitment of resting cells to undergo mitotic division.

0328 EVIDENCE OF CYTOTOXIC T AND B IMMUNOBLASTS IN THE THORACIC DUCT OF RATS BEARING TUMOUR GRAFTS. (Eng.) Denham, S. (Chester Beatty Res. Inst., Surrey, England); Wrathmell, A. B.; Alexander, P. *Transplantation* 19(2):102-114; 1975.

Growth inhibition of target cells was used to measure the lymphocyte cytotoxicity of lymphocytes from the thoracic ducts of Wistar rats immunized with allogeneic or xenogeneic tumors. The tumors studied were the Hooded rat fibrosarcoma, the L5178Y lymphoma and the F56 sarcoma. The immune response to allogeneic cells was due only to radiosensitive thymus-dependent lymphocytes (T cells). Complement-dependent killing of allogeneic cells occurred in immunized animals deprived of T cells. Immunization with xenogeneic cells resulted in complement-dependent and complement-independent cytotoxicity; only complement-dependent killing was seen in animals deprived of T cells. Fractionation of the lymphocytes from the thoracic ducts using density gradient sedimentation revealed the cytotoxic cells to be large lymphocytes or immunoblasts. The occurrence of these cytotoxic cells in lymph coincided with an increase in the number of immunoblasts. The authors propose that the two types of cytotoxicity examined are caused by two populations of immunoblasts, a thymus-dependent and a thymus-independent immunoblast population.

0329 CHANGES IN THE SURFACE MEMBRANE OF LYMPHOCYTES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND HODGKIN'S DISEASE. (Eng.) Mintz, U. (Weizmann Inst. Science, Rehovoth, Israel); Sachs, L. *Int. J. Cancer* 15(2):253-259; 1975.

Lymphocytes were isolated from the peripheral blood of 15 normal persons, 15 patients with chronic lymphocytic leukemia (CLL), 10 patients with Hodgkin's disease and three patients with CLL in remission. The patients with CLL had leukocyte counts of 18,000-140,000/mm^3 and 70-96% lymphocytes. The patients with CLL in remission had leukocyte counts of 6800-8300/mm^3 and 24-25% lymphocytes. The patients with Hodgkin's disease were all newly diagnosed and received no treatment. They had leukocyte counts of 5700-13,600/mm^3 and 3-21% lymphocytes. The 15 normal persons had normal peripheral blood counts. The cells were studied for cap formation and agglutinability by concanavalin A, and for cell attachment to the surface of a Petri dish. The percentages of lymphocytes with a cap were 25-34% for normal persons, 5-9% for CLL patients whether under treatment or not, 1-3% for untreated patients with Hodgkin's disease, and 22-24% for CLL patients in remission. Vinblastine in all cases induced only a 1.5- to 2-fold increase in the percentage of cells with a cap. Agglutinability by concanavalin A was low for normal persons and high for all other patients. Cell attachment to the Petri dish was low for normal persons and Hodgkin's disease patients, intermediate for CLL in remission, and high for patients with CLL. In normal persons, similar results were obtained with peripheral blood lymphocytes that were predominantly T cells and with isolated normal B cells. Compared to lymphocytes from normal persons, those from CLL patients differ in cap formation, agglutinability and cell attachment; those from Hodgkin's disease in cap formation and agglutinability; and those from CLL in remission in agglutinability and cell attachment. These results indicate that there are different surface membrane changes in lymphocytes from patients with CLL, Hodgkin's disease, and CLL in remission. It is suggested that these membrane changes may be useful as an aid in diagnosis and be of value in evaluating the effects of therapy.

0330 CYTOTOXIC EFFECTS OF ANTIGEN- AND MITOGEN-INDUCED T CELLS ON VARIOUS TARGETS. (Eng.) Bevan, M. J. (Salk Inst. Biol. Stud., La Jolla, Calif.); Cohn, M. *J. Immunol.* 114(2):559-565; 1975.

Spleen cells obtained from C57BL/6 and DBA/2 mice were used to assess mitogen-induced cytotoxicity to tumor cells. Concanavalin A (Con A) was added to mouse spleen cell cultures, and two days later the cells were tested for cytotoxicity. Cell lysis was

found to occur only if Con A or phytohemagglutinin P (PHA) was present in the assay medium. The Con A-induced cytotoxic cells were found to be sensitive to complement and anti-θ serum. The effector cells in this case were found to be T cells, and could be induced by H-2 different allo-immunization to specifically lyse targets bearing the immunizing antigen, in the absence of PHA. If PHA was added to the cytotoxic assay system, nonspecific lysis of syngeneic tumor targets was seen. It is proposed that this cytotoxic assay measures only cytotoxic T cells, regardless of their specificity. The proposal is based on the observation that: not all dividing T cells lyse PHA-P815; that the tumor cells used in the cytotoxic assay (P815 and E14) can be lysed by antigen-specific T cells and by nonspecific PHA-revealed lysis; and that small lymphocytes, and T and B cell "blasts" can be easily lysed by T cells directed against H-2 antigens, but not by nonspecific T cell lysis in the presence of PHA. It is suggested that this difference in susceptibility may play a role in *in vivo* tumor rejection.

0331 RELATIONSHIP OF CLONING INHIBITION FACTOR, "LYMPHOTOXIN" FACTOR, AND PROLIFERATION INHIBITION FACTOR RELEASE *IN VITRO* BY MITOGEN-ACTIVATED HUMAN LYMPHOCYTES. (Eng.) Jeffes, E. W. B., III. (Dep. Mol. Biol. Biochem., Univ. California, Irvine); Granger, G. A. *J. Immunol.* 114(1):64-69; 1975.

Human lymphocytes (adenoid and spleen) were stimulated *in vitro* with mitogens, phytohemagglutinin (2000 μg) and concanavalin A (2000 μ) to secrete proliferation inhibitory factor, cloning inhibitory factor, and lymphotoxin. These three activities were demonstrable in the same supernatant; the particular effect observed depended on the concentration of the medium and the type of target cell employed. The medium effects on target cells were: 1) cytotoxicity at high concentrations (1:2 to 1:16 dilutions); 2) growth inhibition at intermediate concentrations (1:16 dilutions); and 3) only temporary growth inhibition at low concentrations (1:32 to 1:256 dilutions). The absolute concentration producing a certain effect varied, depending on the target cell type employed. The sensitivity of the target cells to lymphotoxin paralleled the sensitivity of the cell to each of the other activities; no species specificity was observed. It is suggested that the evidence supports the concept that these inhibiting activities may all be due to the same molecule or family of closely related molecules which has different effects on cells of various concentrations.

0332 IMMUNOLOGIC AND MORPHOLOGIC STUDIES OF T CELL LYMPHOMA. (Eng.) Mann, R. B. (Johns Hopkins Hosp., Baltimore, Md.); Jaffe, E. S.; Braylan, R. C.; Eggleston, J. C.; Ransom, L.; Kaizer, H.; Berard, C. W. *Am. J. Med.* 58(3):307-313; 1975.

Neoplastic cells from a lymph node and the cerebrospinal fluid of a 14-year-old boy with a diffuse malignant lymphoma of the poorly-differentiated lymphocytic type were examined by electron microscopy and in frozen sections and cell suspensions for the presence of surface immunoglobulin and the antigen-antibody-complement (IgMEAC) receptor of B lymphocytes. The presence of the cytophilic antibody (IgGEA) receptor of histiocytes, and the ability to form nonimmune rosettes with sheep RBC, a characteristic of T lymphocytes, were also sought. The majority of neoplastic cells from the lymph node and cerebrospinal fluid formed rosettes with sheep RBC, but lacked surface immunoglobulin and failed to bind IgMEAC or IgGEA. By scanning electron microscopy, the neoplastic cells, although larger in diameter, showed a surface architecture similar to that of normal lymphocytes with a varying number of surface microvilli. Thus, unlike the majority of previously studied lymphomas in adults, which have been of B cell origin, the malignant cells in this case appeared to be of the thymic type.

0333 CLINICAL IMPORTANCE OF LYMPHOBLASTS WITH T MARKERS IN CHILDHOOD ACUTE LEUKEMIA. (Eng.) Sen, L. (St. Jude Child. Res. Hosp., Memphis, Tenn.); Borella, L. *N. Engl. J. Med.* 292(16):828-832; 1975.

The possible correlations of age, sex, initial white-cell count, and mediastinal enlargement with the presence of sheep erythrocyte receptors or surface immunoglobulin on the bone-marrow blast cells were studied in 48 children with untreated acute lymphocytic leukemia. Surface immunoglobulins were assayed by direct immunofluorescence microscopy and sheep erythrocyte receptors were detected by the ability of the blast cells to form rosettes on incubation with sheep erythrocytes. None of the patients had detectable blast cell surface immunoglobulin, while the blast cells from 11 of the patients formed erythrocyte rosettes; the percentage of lymphoblasts with sheep erythrocyte receptors varied from 2-76%, with a median of 50%. The patients whose cells formed erythrocyte rosettes were all boys, whereas the group without erythrocyte receptors had nearly equal numbers of boys and girls. In addition, the median age of the children with erythrocyte rosette-forming cells was nine yr, while that of the children without rosette-forming cells was 4.5 yr. All but one of the eight children with radiographic evidence of a thymic mass had rosette forming blast cells, and the median initial white cell count in this group was 52,000 (compared with 13,000 in the children without rosette-forming cells). Thus, the presence or absence of sheep erythrocyte receptors on the bone-marrow leukemic blast cells appears to distinguish two forms of acute lymphocytic leukemia, each having distinct clinical features. The data suggest that sheep erythrocyte receptors on leukemic blast cells have clinical and biologic implications.

0334 T & B LYMPHOCYTES IN THYMUS OF SJL/J MICE. (Eng.) Ben-Yaakov, M. (Dep. Chem. Immunol., Weizmann Inst. Sci., Rehovot, Israel); Haran-Ghera, N. *Nature* 255(5503):64-66; 1975.

The thymus cell population pattern of SJL/J mice was investigated using membrane antigenic markers and functional criteria to study previously reported age-dependent changes of immunoresponsiveness in this

strain. Using a cytotoxic test, a progressive decrease in the percentage of θ antigen-bearing cells was noted from four months on, and a progressive increase in the percentage with the H-2 alloantigen was noted. Starting at two months of age, progressive increases in the percentages of cells bearing IgG and IgM immunoglobulins were noted reaching a 50% incidence of IgG and 22% IgM at five months of age. A double labeling technique showed that θ antigen and immunoglobulin were not carried at the same time. The B cells present in the thymus were able to resynthesize immunoglobulins *in vitro* following treatment with proteolytic enzymes. The age-related increase in the H-2 population was correlated with an increased capacity of the T cells to induce a GVH response. The authors suggest that the θ-positive, high H-2 immunocompetent population of cells is enriched in the nine-month-old thymus and reduced in the 15-month-old thymus. In contrast with other strains, the SJL/J mouse thymus cells demonstrated an age-related increase in colony-forming ability. The authors suggest that the susceptibility of SJL/J mice to carcinogen-induced B lymphatic leukemia may be correlated with the capacity of B cells to home in the thymus of this strain.

0335 CHARACTERIZATION OF THE LYMPHOCYTE SURFACE RECEPTORS FOR CON A AND PHA. (Eng.) Henkart, P. A. (Natl. Cancer Inst., Bethesda, Md.); Fisher, R. I. *J. Immunol.* 114(2):710-714; 1975.

Human peripheral blood lymphocytes from normal donors were iodinated with ^{131}I by the lactoperoxidase method to study the molecular nature of the cell surface receptors for concanavalin A (Con A) and phytohemagglutinin (PHA). Following iodination, living lymphocytes were separated by density gradient centrifugation, and their membranes were solubilized. Aliquots of the membrane were incubated with Con A and PHA mitogens and antimitogen antibody. The precipitates formed were subjected to gel electrophoresis for analysis of protein binding by the mitogens. The patterns of protein binding of Con A and PHA were quite similar, but Con A bound two times the amount of labelled protein as PHA did. The binding patterns showed multiple peaks indicating that many surface proteins, ranging in molecular weight from 43,000 to 68,000 daltons, bound these mitogens. The patterns obtained from the freshly drawn lymphocytes were comparable to those obtained from long-term lymphoid tissue culture cell lines. The results indicate that the heterogeneity of the receptors binding Con A and PHA is not due exclusively to lymphocyte heterogeneity, and that no specific cell surface receptor is responsible for the binding of these mitogens.

0336 INTRACELLULAR POTENTIAL IN NORMAL AND LEUKEMIC LYMPHOCYTES. (Eng.) Malofiejew, M. (Med. Sch., Bialystok, Poland); Kostrzewska, A.; Kowal, E. *Acta Haematol. Pol.* 53(3):138-144; 1975.

The electrobiological characteristics of peripheral lymphocytes from normal subjects and patients with chronic lymphatic leukemia were compared. The leukemic lymphocytes had a significantly lower intracellular potential than normal lymphocytes (-5.6 ± 0.1 mV *versus* -7.4 ± 0.2 mV). Whereas the intracellular potential of normal lymphocytes showed a linear dependence on the potassium ion concentration of the medium, the potential of leukemic lymphocytes increased rapidly at low potassium concentrations and remained unchanged at high concentrations. Low chlorine ion concentrations caused a decrease in the intracellular potential of normal lymphocytes but had no effect on that of leukemic lymphocytes. It is concluded that the different electrobiological properties of leukemic lymphocytes might result from different mechanisms conditioning or controlling ion transport through the cell membrane.

0337 THE INFLUENCE OF DIFFERENT ISOLATION PROCEDURES AND THE USE OF TARGET CELLS FROM MELANOMA CELL LINES AND SHORT-TERM CULTURES ON THE NON-SPECIFIC CYTOTOXIC EFFECTS OF LYMPHOCYTES FROM HEALTHY DONORS. (Eng.) de Vries, J. E. (Netherlands Cancer Inst., Amsterdam, Holland); Meyering, M.; van Dongen, A.; Rümke, P. *Int. J. Cancer* 15(3): 391-400; 1975.

The factors influencing nonspecific cytotoxic effects on lymphocytes were investigated. Ficoll-Hypaque lymphocytes (FHL) showed more frequent and stronger cytotoxic effects on melanoma cells from short-term cultures and cell lines than plasmogel nylon-wool column lymphocytes (PNL). Both FHL and PNL showed cytotoxic effects on melanoma cells from cell lines more than from short-term cells. There was an increase in susceptibility of melanoma cells for PNL from healthy donors after prolonged culture (eight months). The multiplicity of the cell line cells during the test period was 38% compared to a -13% in the short-term culture. FHL and PNL from five healthy donors showed a positive lymphocyte cytotoxicity score (LCS) on HBT-3, 2T and NKI-4 target cells with FHL giving the highest score. When human RBC sensitized with rabbit anti-human antiserum and human complement (EAC) were separated from 25 donors PNL showed a significantly lower EAC-rosette forming percentage than FHL cells. Stained smears of FHL and PNL showed the mean percentage of monocytes in FHL were significantly higher than in PNL. In general, the cytotoxic effects of lymphocytes from healthy donors may not have any relevance with respect to an anti-tumor response.

0338 LYMPHOCYTE TRANSFORMATION IN LEUKEMIC SERUM. (Eng.) Humphrey, G. B. (Univ. Oklahoma Health Sci. Cent., Oklahoma City,; Peterson, L.; Whalen, M.; Parker, D. E.; Lankford, J.; Krivit, W.; Nesbit, M. *Cancer* 35(5):1341-1345; 1975.

Pokeweed mitogen (PWM)-induced transformation of normal lymphocytes in the presence of sera from normal children or children in various phases of leukemia was examined to determine if leukemic sera contained factors which affected lymphocyte transformation. Transformations were lower in the presence of leukemic blastic sera (WBC count greater than 50,000/mm^3) than in leukemic (WBC count less than 50,000/mm^3), leukemic relapse (greater than 5% blasts in bone marrow) or leukemic remission sera,

which were not different from normal sera. Increasing the sera from 20% to 60% of the medium resulted in greater inhibition of transformation. In one experiment with two normal and two leukemic sera, the leukemic sera were inhibitory throughout a range of 1-20 µl/ml PWM indicating that binding of PWM by serum components is not responsible for the inhibition. Twenty-eight of 38 sera were assessed for cytotoxicity and no correlation between cytotoxicity and transformation inhibition was noted. The results indicate the presence of a transformation inhibitor in sera from patients with active leukemia. The authors propose that measurement of this inhibition may be useful in the diagnosis and treatment of leukemia.

0339 THE DOSE-RELATED EFFECT OF TUMOUR EXTRACT ON THE *IN VITRO* MIGRATION OF LEUCOCYTES FROM PATIENTS WITH RENAL CARCINOMA. (Eng.) Kjaer, M. (Division of Clinical Immunology, Medical Department TA, Rigshospitalet, University Hospital of Copenhagen, Denmark). *Eur. J. Cancer* 11(4):281-289; 1975.

Peripheral blood leukocytes were obtained from 43 patients with renal carcinoma (17 with distant metastases), four patients with benign renal disease, seven patients with cancer of non-kidney origin, 45 patients with various benign disease and 27 healthy blood donors to test the effects of extracts from normal renal tissue and allogeneic hypernephroma tissues on leukocyte migration. The capillary tube leukocyte migration technique (LMT) was used. Eighteen of 26 patients with renal carcinoma without metastases showed altered reactivity towards tumor extract at concentrations of 300 and 400 µg protein per ml. Leukocyte reactivity at fixed extract concentrations were not different in any of the groups studied. Significant differences between leukocyte reactivity of renal carcinoma patients without metastases and controls in the position and dispersion of distribution were seen at allogeneic tissue extract concentrations of 50, 100, 300, and 400 µg protein per ml. The results indicate that leukocytes obtained from patients with renal carcinoma without distant metastases have an altered, dose-related *in vitro* reactivity towards allogeneic hypernephroma tissue extract.

0340 *IN VITRO* IMMUNOLOGICAL STUDIES ON EAST AFRICAN CANCER PATIENTS. III. SPONTANEOUS ROSETTE FORMATION BY CELLS FROM BURKITT LYMPHOMA BIOPSIES. (Eng.) Gross, R. L. (Western General Hosp., Crewe Road, Edinburgh, EH4, 2XU, Scotland); Steel, C. M.; Levin, A. G.; Singh, S.; Brubaker, G. *Int. J. Cancer* 15(1):139-143; 1975.

Biopsies from nine African children with Burkitt's lymphoma (five boys and four girls, aged 3-9 yr) were tested for the capacity of tumor cells to form spontaneous rosettes with sheep red blood cells (SRBC). The biopsies were examined fresh and/or after culture *in vitro* for up to 48 hr. The results were completely negative in only one case. With the other eight tumors, 3.7-38% of the cells formed rosettes with large numbers of adherent SRBC. When the samples from three patients were examined at intervals, the percentage of rosettes tended to increase. The cumulative evidence is strong that rosette-forming lymphocytes are almost always T-cells. It is therefore suggested that T-lymphocytes in Burkitt's lymphoma represent a cell-mediated host defense agains proliferating B-cells.

0341 MACROPHAGES ACTIVATED *IN VITRO* WITH LYMPHOCYTE MEDIATORS KILL NEOPLASTIC BUT NOT NORMAL CELLS. (Eng.) Piessens, W. F. (Harvard Med. Sch., Boston, Mass.); Churchill, W. H., Jr.; David, J. R. *J. Immunol.* 114(1):293-299; 1975.

The effects of *in vitro* activation with lymphocyte mediators on the cytotoxic activity of normal guinea pig macrophages (against syngeneic tumor cells) were studied. Randombred Hartley and inbred Sewall Wright strain 2 guinea pigs were sensitized by the injection of O-chlorobenzoyl bovine α-globulin (OCB-BGG) into each footpad and the dorsal nuchal dermis. Lymphocyte supernatants were prepared from these animals 14-21 days later. Macrophage monolayers obtained from normal strain 2 guinea pigs were incubated for three days with the appropriate lymphocyte supernatant (activated macrophages) or with control supernatants. The cytotoxicity of these macrophages against line 1 hepatoma cells of strain 2 guinea pigs, MCA-25 fibrosarcoma cells, strain 2 embryonic fibroblasts, and strain 2 kidney cells was determined. The activated macrophages exhibited an enhanced ability to kill the syngeneic line 1 hepatoma and MCA-25 fibrosarcoma cells; they exhibited no enhanced ability to kill either nonmalignant syngeneic fibroblasts or kidney cells. Since the OCB-BGG antigen used to prepare the mediator-rich supernatants was unrelated to the tumor cell types killed by the activated macrophages, this type of macrophage-mediated cytotoxicity appears to be nonspecific but is restricted to cells with malignant growth capacities. Lymphocyte mediators may play an important role *in vivo* in the rejection of tumors by activating normal macrophages and rendering them cytotoxic for tumor cells.

0342 IMPAIRED MACROPHAGE FUNCTION IN FRIEND VIRUS LEUKEMIA: RESTORATION BY STATOLON. (Eng.) Levy, M. H. (Dep. Microbiol., Thomas Jefferson Univ., Philadelphia, Pa.); Wheelock, E. F. *J. Immunol.* 114(3):962-965; 1975.

The phagocytic and migratory capacities of peritoneal macrophages obtained from Friend virus (FV)-leukemic DBA/2 mice were determined, and the effects of *in vivo* and *in vitro* treatment with statolon were measured. Leukemic macrophages had decreased migratory and phagocytic functions compared to controls. Statolon (which has been shown to suppress FV erythroleukemia *in vivo*), in concentrations of 31.25 to 500 µg/ml *in vitro*, restored the phagocytic and migratory capacities of the leukemic macrophages to normal. The higher levels of statolon increased interferon *in vitro*, but did not at the lowest concentrations used. The restoration of macrophage function was paralleled by a return of humoral immune competence. The results indicate that the restoration of macrophage function in FV-leukemic mouse macrophages was caused by levels of statolon, which

did not affect interferon and is most probably a direct effect. The authors suggest that macrophages may play a major role in the pathogenesis of FV leukemia, and its suppression by statolon.

0343 POSSIBLE ANTINEOPLASTIC AGENTS I. (Eng.) De, A. U. (Dept. Pharm., Jadavpur Univ., Calcutta, India); Pal, D. *J. Pharm. Sci.* 64(2):262-266; 1975.

0344 INDUCTION OF PERMANENTLY PROLIFERATING HUMAN LYMPHOBLASTOID LINES BY *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE. (Eng.) Henderson, E. E. (Dept. Microbiol., Univ. Chicago, Ill.); Norin, A. J.; Strauss, B. S. *Cancer Res.* 35(2):358-363; 1975.

0345 THE ROLE OF SERUM FACTORS IN THE ACCELERATION BY FREUND'S COMPLETE ADJUVANT OF THE GROWTH OF TRANSPLANTED MURINE LEUKEMIC CELLS. (Eng.) Byfield, P. E. (Univ. California Los Angeles Sch. Med., Harbor General Hosp., Torrance); Finklestein, J. Z.; Tittle, K. L.; Hsi, C.; Imagawa, D. T. *Cancer Res.* 35(2):409-414; 1975.

0346 BLOCKING ANTIGEN-ANTIBODY COMPLEXES ON THE T-LYMPHOCYTE SURFACE IDENTIFIED WITH DEFINED PROTEIN ANTIGENS. II. LYMPHOCYTE ACTIVATION DURING THE *IN VITRO* RESPONSE. (Eng.) Kontiainen, S. (Imp. Cancer Res. Fund, Univ. Coll., London, England). *Immunology* 28(3):535-542; 1975.

0347 SCREENING, ISOLATION AND SOME PROPERTIES OF MOUSE TUMOR CELL AGGLUTININ. (Eng.) Oishi, K. (Inst. Appl. Microbiol., Univ. Tokyo, Japan); Aida, K. *Agric. Biol. Chem.* 39(1):183-191; 1975.

0348 ENZYMATIC ACTIVATION AND TRAPPING OF LUMINOL-SUBSTITUTED PEPTIDES AND PROTEINS. A POSSIBLE MEANS OF AMPLIFYING THE CYTOTOXICITY OF ANTI-TUMOR ANTIBODIES. (Eng.) Parker, C. W. (Washington Univ. Sch. Med., St. Louis, Mo.); Aach, R. D.; Philpott, G. W. *Proc. Natl. Acad. Sci. USA* 72(1):338-342; 1975.

0349 ULTRASTRUCTURAL EVIDENCE FOR DESTRUCTION IN THE HALO NEVUS. (Eng.) Jacobs, J. B. (St. Vincent Hosp., Worcester, Mass.); Edelstein, L. M.; Snyder, L. M.; Fortier, N. *Cancer Res.* 35(2):352-357; 1975.

0350 HOST RESPONSES WITHIN SOLID TUMORS. I. MONOCYTIC EFFECTOR CELLS WITHIN RAT SARCOMAS. (Eng.) Haskill, J. S. (McGill Univ. Cancer Res. Unit, Montreal, Canada); Proctor, J. W.; Yamamura, Y. *J. Natl. Cancer Inst.* 54(2):387-393; 1975.

0351 DEVELOPMENT OF CONCOMITANT IMMUNITY IN MICE BEARING THE WEAKLY IMMUNOGENIC LINE 1 LUNG CARCINOMA. (Eng.) Yuhas, J. M. (Biol. Div., Oak Ridge Natl. Lab., Tenn.); Pazmino, N. H.; Wagner, E. *Cancer Res.* 35(1):237-241; 1975.

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0374 IgG HALF-MOLECULES: CLINICAL AND IMMUNOLOGIC FEATURES IN A PATIENT WITH PLASMA CELL LEUKEMIA. (Eng.) Spiegelberg, H. L. (Scripps Clin. Res. Found., La Jolla, Calif.); Heath, V. C.; Lang, J. E. *Blood* 45(3):305-314; 1975.

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0395 TWO POPULATIONS OF LYMPHOCYTES IN A CAT. (Eng.) Mackey, L. J. (Dept. Vet. Pathol., Univ. Glasgow, Scotland); Jarrett, W. F. H.; Coombs, R. R. A. *Vet. Rec.* 96(2):41; 1975.

0396 AGGLUTINATION OF 2,4-DINITROPHENYL-TAGGED NORMAL HUMAN LEUKOCYTES BY CONCANAVALIN A: POSSIBLE RELATIONSHIP TO THEIR ABILITY TO EVOKE PRODUCTION OF LEUKEMIA-ASSOCIATED ANTIBODIES. (Eng.) Barth, R. F. (Univ. Kansas Med. Cent., Kansas City); Madyastha, K. R.; Madyastha, P. R. *J. Natl. Cancer Inst.* 54(1):73-76; 1975.

See also:
* (Rev): 0008, 0009, 0010, 0011, 0012, 0038, 0039, 0040, 0041, 0042, 0043
* (Chem): 0063, 0116, 0125
* (Phys): 0180
* (Viral): 0200, 0201, 0208
* (Path): 0400, 0413, 0419, 0433
* (Epid-Biom): 0520

0397 VINYL-CHLORIDE-INDUCED LIVER DISEASE: FROM IDIOPATHIC PORTAL HYPERTENSION (BANTI'S SYNDROME) TO ANGIOSARCOMAS. (Eng.) Thomas, L. B. (Natl. Cancer Inst., Bethesda, Md.); Popper, H.; Berk, P. D.; Selikoff, I.; Falk, H. *N. Engl. J. Med.* 292(1):17-22; 1975.

Hepatic and splenic tissues derived from 20 workers with prolonged (2-18 yr) industrial exposure to vinyl chloride were examined histologically. The tissue specimens included 20 samples (eight taken at autopsy and 12 at biopsy) from 15 patients with angiosarcoma, and six biopsy samples (five surgical and one needle-biopsy specimen) from five patients with hepatic fibrosis without known angiosarcoma; the splenic samples were obtained surgically in three cases and at autopsy in two cases. The tissue specimens confirmed the diagnosis of hepatic angiosarcoma in 15 patients and showed a peculiar pattern of progressive portal-tract, inconspicuous intralobular, and conspicuous capsular fibrosis in the five patients without angiosarcoma, in all seven tumor-free portions of the liver obtained from the angiosarcoma patients, and in two tumor-free biopsies from patients subsequently found to have angiosarcoma. The fibrosis was accompanied by splenomegaly, and hypertrophy and hyperplasia of both hepatocytes and hepatic and splenic mesenchymal cells were also seen. The histological similarity to chronic inorganic arsenical poisoning, in which angiosarcomas also occur, and to idiopathic portal hypertension (Banti's syndrome) suggests that the latter syndrome at times results from unknown toxic, possibly environmental, chemicals.

0398 CYTOLOGICAL AND CYTOGENETICAL STUDIES ON BRAIN TUMORS. V. PREFERENTIAL LOSS OF SEX CHROMOSOMES IN HUMAN MENINGIOMAS. (Eng.) Zankl, H. (Dep. Hum. Genet., Univ. Saarland, Homburg, West Germany); Seidel, H.; Zang, K. D. *Humangenetik* 27(2): 119-128; 1975.

Chromosomal analysis was performed on human meningiomas to determine whether the sex chromosomes were lost. Of 122 primary cultures of tumors studied, 88 were of female, and 24 were of male origin. Of the female tumors, 12 were deficient in one to three group C chromosomes, suggesting the loss of an X-chromosome. Of the 24 male tumors, all were deficient in one or two group G chromosomes, suggesting loss of a Y chromosome. X chromosome loss was determined by examination of sex chromatin in interphrase nuclei; also, one case was determined by Giemsa banding. Y chromosome was identified by its specific morphology and its noninvolvement in satellite associations. In the eight most recent cases the loss of male sex chromosome could be ascertained by the conspicuous absence of Y fluorescence in interphase nuclei and in metaphase plates after fluorescence staining. In eight of the 12 group C chromosome-deficient tumors, less than 8% Barr bodies were found. One tumor had 12% and three had over 20% Barr bodies. By comparison with control cultures, it was determined that three tumors had both X chromosomes, eight had lost one X, and one had lost one X chromosome in part of its cells. Of all 122 tumors, only four had hypodiploidy without loss of a G chromosome. In one of these, loss of an X chromosome was confirmed by Giemsa banding. In 18 male tumors missing one G chromosome, fluorescence staining showed that the Y chromosome was definitely present in at least four. In the six tumors missing both G chromosomes, the Y chromosome was missing. This was confirmed in four tumors by fluorescence staining. The results indicate that the loss of sex chromosomes is relatively frequent in human meningiomas.

0399 PRIMARY RHABDOMYOSARCOMA OF THE CEREBRUM. (Eng.) Min, K.-W. (Veterans Adm. Hosp., Houston, Tex.); Gyorkey, F.; Halpert, B. *Cancer* 35 (5):1405-1411; 1975.

A case report of a rhabdomyosarcoma of the cerebrum in a 48 yr-old Negro male initially presenting a left hemiparesia and exhibiting general confusion was described. A ventriculogram showed a shift of the entire ventricular system and some depression and backward displacement of the anterior horn of the right ventricle. A right frontotemporal craniotomy was performed and a tumor mass was excised from the right frontal lobe inferiorly. Four months later an additional craniotomy was performed, and a recurrent tumor was excised; a right carotid arteriogram performed two months later revealed a recurrent tumor in the right frontal lobe. The tumor continued to enlarge until death. The first surgical specimen exhibited a pleomorphic pattern, areas of undifferentiated cells intermingled with giant cells, frequent mitotic figures, and a highly vascular appearance; the dura mater was not microscopically involved. The morphological features of the second surgical specimen were identical to the first, as was the recurrent brain tumor revealed at autopsy, at which time the tumor grossly appeared to infiltrate the dura mater. In addition, two circumscribed metastatic nodules were located in the liver. The morphological features diagnostic of a rhabdomyosarcoma were especially frequent strap cells among undifferentiated mesenchymal cells and cells with unequivocal cytoplasmic cross-striations. Although microscopic sections showed no frank vascular invasion, the recurrent tumor grew toward the base of the skull along the inner aspect of the dura mater; this provided an excellent chance for vascular invasion and metastasis. The high vascularity of the tumor may suggest that the tumor cells penetrated through the vascular wall and entered into the vascular system. The authors believe that the case represents a unique example of primary cerebral rhabdomyosarcoma with extracranial metastasis.

0400 CONSECUTIVE PRIMARY CARCINOMAS OF THE BREAST. (Eng.) McCredie, J. A. (Dep. Surg., Univ. West. Ontario, London, Canada); Inch, W. R.; Alderson, M. *Cancer* 35(5):1472-1477; 1975.

The incidence of contralateral carcinoma in patients with breast carcinoma treated for cure, and the factors influencing survival were studied. The incidence of consecutive breast cancers in patients given prophylactic postoperative radiotherapy was also studied. Patients records were reviewed, separate life tables were computed for each age group,

and cumulative probabilities and their standard errors were calculated and tested for statistical significance at the 5% level; regression lines and correlation coefficients were then determined. The study included 369 patients treated by operation and not irradiated, and 1120 treated by radical mastectomy and given prophylactic postoperative radiotherapy. Patients in the latter group were younger; those developing nonsynchronous breast carcinomas were significantly younger, although the cumulative probability of developing a consecutive nonsynchronous carcinoma of the breast at different ages and at all ages did not differ between the groups. Recurrence was 52% in the women with single tumors and 51% in those with bilateral nonsynchronous tumors; the most common tumor was the infiltrating duct carcinoma with fibrosis (88%). The possibility of developing a nonsynchronous consecutive carcinoma of the breast was about 1% per year, and remained constant over 20 yr. In contrast to other studies, no significant age difference was found, although it was noted that women developing nonsynchronous consecutive carcinomas were six yr younger than those with single tumors. The prognosis was poor when there was a short interval and second tumors. The recurrence rate was the same in those with single and bilateral carcinomas of the breast, while women with invasive lobular carcinomas had a 30% chance of developing a carcinoma of the opposite breast. The high incidence (50%) of positive nodes in those with a second tumor suggests a late diagnosis. The authors suggest that new lesions and any region with signs of malignancy on mammography be excised and examined microscopically, and that a contralateral prophylactic subcutaneous mastectomy be performed in all women with lobular carcinoma.

0401 CHOLANGIOCARCINOMA IN A PATIENT PREVIOUSLY GIVEN THOROTRAST. (Eng.) Johnson, P. K. (Palo Alto Med. Clin., Calif.); Babb, R. R. *Am. J. Dig. Dis.* 20(4):384-390; 1975.

A case report of a 48-yr-old woman with a thorotrast-induced cholangiocarcinoma is presented. To date, more than 100 cases of liver cancer have been reported in patients who had received thorotrast, a colloidal solution of thorium dioxide. Thorotrast has a latency period of 22 yr. In 1952, the patient received 20 cc of thorotrast *via* cerebral arteriography as part of a neurological examination. In 1974, she complained of fatigue, anorexia, and upper-abdominal pain of three months duration. A peripheral smear revealed variation in RBC size with occasional teardrop cells, poikilocytosis, nucleated RBC, and frequent Howell-Jolly bodies. Lymphocytes were atypical and plasmacytoid features were noted. Liver function tests showed normal total bilirubin and SGOT values, but alkaline phosphatase was 250 IU (normal < 85). A plain film of the abdomen showed densities within the liver, lymph nodes, and spleen consistent with thorotrast deposition. Filling defects in the liver and an enlarged spleen were noted on the ^{99m}Tc sodium pertechnetate liver scan. At laparotomy, extensive cancer of the liver was found. Resection was impossible. Multiple liver biopsies showed areas ranging from normal liver tissue to areas of fibrosis, necrosis, and bile duct proliferation. Crystalline material consistent with thorotrast was distributed randomly throughout the liver. Patients who received thorotrast in the late 1940s and 1950s are now at risk, and the association with liver cancer should be remembered.

0402 HUMAN CHRONIC MYELOGENUOUS LEUKEMIA CELL-LINE WITH POSITIVE PHILADELPHIA CHROMOSOME. (Eng.) Lozzio, C. B. (Univ. Tennesse Mem. Res. Cent., Knoxville); Lozzio, B. B. *Blood* 45(3):321-334; 1975.

The origin, culture method and characteristics of the first permanent cell-line available with a persistent positive Philadelphia (PH') chromosome after prolonged *in vitro* cultivation were presented. The cell-line was derived from a pleural effusion of highly undifferentiated cells from a female at the terminal stages of an acute blastic crisis of chronic myelogenous leukemia (CML). Pleural fluid was collected with heparin. Aliqouts containing 15-20 million cells each were diluted with Eagle's minimal essential medium plus additional amino acids and 15% fetal bovine serum. Cultures were incubated at 37 C in humidified atmosphere with constant flow of 5% CO_2 in air. Cells grew as free-floating suspensions in liquid medium. The characteristics of the CML cell-line are: active proliferation upon incubation in culture media; the appearance of mononucleated cells as undifferentiated blasts; the presence of the Ph' chromosome and a long acrocentric marker plus aneuploidy in the karyotype; no production of immunoglobulins; and lack of mycoplasma, Epstein-Barr virus or herpes virus-like particles. In addition, CML cells have no alkaline phosphatase or myeloperoxidase activity and do not engulf inert particles. Cultured CML cells provide a constant source of a specific antigen. This cell-line represents a unique source of human CML cells with meaningful indicators of malignancy for clinical and experimental studies.

0403 NONRANDOM CHROMOSOMAL ABNORMALITIES IN HEMATOLOGIC DISORDERS OF MAN. (Eng.) Rowley, J. D. (Dep. Med., Univ. Chicago, Ill.). *Proc. Natl. Acad. Sci. USA* 72(1):152-156; 1975.

Nonrandom chromosomal abnormalities were studied in cells obtained from 21 patients with various hematologic disorders, such as chronic myelogenous leukemia, acute leukemia, polycythemia, anemia, and neutropenia. Chromosomes were prepared from bone marrow cells and stained with quinacrine mustard when in metaphase. Individual C group chromosomes were identified by their banding pattern. Consistent patterns of chromosomal abnormalities were confirmed by analysis of 29 case reports from other laboratories. The most common abnormality was an additional number 8 chromosome, found in more than 50% of the patients studied. An additional number 9 chromosome and the loss of all or part of a number 7 occurred significantly more often than would be expected by chance. Phytohemagglutinin-stimulated lymphocytes from these patients showed a normal karyotype. The chromosome change in the marrow cells, therefore, represents a somatic mutation superimposed on the individual's normal chromosome pattern. It is proposed that specific human chromosomal abnormalities may be related to different specific etiologic agents.

0404 *DE NOVO* APPEARANCE OF THE Ph^1 CHROMOSOME IN A PREVIOUSLY MONOSOMIC BONE MARROW (45,XX,-6): CONVERSION OF A MYELOPROLIFERATIVE DISORDER TO ACUTE MYELOGENOUS LEUKEMIA. (Eng.) Kohn, G. (Hadassah-Heb. Univ. Med. Cent., Jerusalem, Israel); Manny, N.; Eldor, A.; Cohen, M. M. *Blood* 45(5):653-657; 1975.

A case report of a 38-yr old woman with myeloproliferative disease is presented. Original investigations revealed a bone marrow karyotype monosomic for group C chromosome (45,XX,-6). Further investigation, however, revealed the *de novo* development of a Ph^1-positive clone in the original aneuploid cell line during blastic crisis (acute myelocytic leukemia). In December, 1972, the patient reported fatigue of one month duration, bleeding tendency, anemia, and thrombocytopenia. Examinations revealed marked pallor, a mild purpuric rash of the extremities, and a palpable spleen 2 cm below the left costal margin. A peripheral blood smear revealed microcytic, hypochromic RBC with anisocytosis, poikilocytosis, target cells, a few nucleated RBC, a shift to the left in WBC series, and bizarre-looking thrombocytes. The bone marrow aspirates were hypercellular with 29% myeloblasts, diminished megakaryocyte numbers, and mild megaloblastic changes in erythroid series. Alkaline-resistant hemoglobin was 2% (normal, 1.0%) and a leukocyte alkaline phosphatase core of 72 (normal, 40-100). These findings suggested a myeloproliferative disorder or smoldering type of acute myeloblastic leukemia. The patient remained stable for six months but was then readmitted with a sudden deterioration of her condition. Reinvestigation of bone marrow showed the presence of the Ph^1 chromosome in the previously aneuploid cell line (45,XX,-6,-22,+Ph^1). Thus, this case differs from those previously described in that the Ph^1 chromosome appeared *de novo* during the development of frank, acute myelogenous leukemia.

0405 RELATION BETWEEN CHROMOSOMES AND MITOTIC ACTIVITY IN ACUTE MYELOID LEUKEMIA. (Eng.) Brandt, L. (Univ. Hosp., S-221 85 Lund, Sweden); Mitelman, F.; Sjögren, U. *Hereditas* 79(2):305-306; 1975.

To examine the relation between mitotic activity and the malignant properties of granulopoietic precursor cells, the mitotic indices of 2,300-13,100 of these cells were determined in ten acute myeloid leukemia (AML) patients, where the bone marrow had a uniform karyotype, and in 15 normal controls. Karyotyping was performed by a trypsin-Giemsa banding technique. Six of the ten patients had 100% abnormal cells and four had 100% normal diploid cells. Mitotic indices of the AML patients were low. There was no indication that cells with normal karyotypes had a more "normal" mitotic activity than those with abnormal karyotypes. Therefore, if low mitotic activity is a sign of "leukemicness" of the granulopoietic precursor cells, then mitotic abnormality is not related to an abnormal chromosomal constitution of these cells.

0406 PHEOCHROMOCYTOMAS OCCURRING IN 3 MEMBERS IN A FAMILY. (Eng.) Sato, T. (Tohoku Univ. Sch. of Medicine, Sendai, Japan); Sato, Y.; Sakuma, H.; Kobayashi, K.; Miura, Y.; Yoshinaga, K.; Watanabe, H. *Tohoku J. Exp. Med.* 115(3):263-269; 1975.

The case reports of three siblings with bilateral adrenal pheochromocytomas are presented. The rate of tumor growth, the stage of asymptomatic or chemical pheochromocytoma, and the importance of urinary catecholamine assay for detection and diagnosis of this disorder are emphasized. In each case, years of slightly high catecholamine output preceded tumor identification. Urinary catecholamine assay values before tumor removal for Case 1, a 24-yr-old male; Case 2, a 26-yr-old female; and Case 3, a 32-yr-old female were 165 μg of epinephrine and 3,200 μg of norepinephrine; 74 μg epinephrine and 1,054 μg norepinephrine; and 14 μg epinephrine and 250 μg norepinephrine, resp. (Normal values of epinephrine and norepinephrine are 0-10 μg and 10-50 μg/day, resp.) As familial occurrence of pheochromocytoma has been established, it is suggested that members of a family with a history of such tumors undergo periodic urinary catecholamine assays.

0407 A FAMILY WITH ATYPICAL COLONIC POLYPOSIS AND GASTRIC CANCER: A THREE-DECADE FOLLOW-UP. (Eng.) Lindberg, B. (Dep. Surg. III, Univ. Göteborg, Sweden); Kock, N. G. *Cancer* 35(1):255-259; 1975.

A three-decade followup of a family in whom multiple colonic polyps were found in five of eleven members of the third generation is presented. Polyps were found in a male and a female of this generation and the three others, two males and one female, suffered from colorectal cancer. The clinical picture and the pathological findings differed from those of familial polyposis. In the previous generation, two cases of gastric cancer, including the father and another male, and one case of colonic cancer in a female occurred. The mother and her siblings were over 70 yr old without symptoms of intestinal disease and were omitted from the pedigree. The high incidence of colonic polyps and cancer occurring in this family, the malignant degeneration that had taken place in three of the cases, and the fact that male twins of the third generation both had cecal cancer in addition to polyps in other parts of the colon, support the suggestion that the polyposis was hereditary. The mode of genetic transmission cannot be determined without an extended followup.

0408 BILATERAL ADRENAL MEDULLARY HYPERPLASIA IN MULTIPLE ENDOCRINE NEOPLASIA, TYPE 2. THE PRECURSOR OF BILATERAL PHEOCHROMOCYTOMA. (Eng.) Carney, J. A. (Mayo Clin.. Rochester, Minn.); Sizemore, G. W.; Tyce, G. M. *Mayo Clin. Proc.* 50(1):3-10; 1975.

A case of bilateral hyperplasia of the adrenal medulla in an asymptomatic 12 year-old girl is documented. The patient, with multiple endocrine neoplasia, type 2, had high urinary levels of vanillylmandelic acid, suggesting pheochromocytoma; she also had hyperthyroidism. Her mother and maternal aunt and uncle had bilateral pheochromocytoma (metastic in the former two). Bilateral adrenalectomy was performed. Adrenal glands from six girls, aged 10 to 13 yr, were

also studied. The adrenal glands were studied by light and electron microscopy and catecholamine extraction. Diffuse, non-nodular adrenal medullary hyperplasia was present. It was characterized by increased medullary mitotic activity, decreased corticomedullary ratio, increased total adrenal weight, and increased total catecholamine content (left adrenal). It is suggested that diffuse hyperplasia of the adrenal medulla may be the precursor of pheochromocytoma in patients with this syndrome.

0409 ENHANCING EFFECT OF HYDROCORTISONE ON HEMATOGENOUS METASTASIS OF EHRLICH ASCITES TUMOR IN MICE. (Eng.) Kodama, M. (Aichi Cancer Res. Inst., Nagoya, Japan); Kodama, T. *Cancer Res.* 35(4): 1015-1021.

The effect of hydrocortisone on blood-borne tumor metastasis was investigated in an i.v. inoculation experiment with three cell lines or Ehrlich ascites tumors: hypotetraploid clone 1, hypotetraploid stock, and hyperdiploid stock. Administration of hydrocortisone before tumor inoculation (5 x 10^6 tumor cells *via* the tail vein of female Swiss/ICR mice) resulted in creased tumor take, reduced mean survival time, and concentration of tumor metastasis in a specific organ (e.g., lung metastasis for Ehrlich hypotetraploid clone 1 tumor, liver metastasis for hypotetraploid stock and hyperdiploid stock tumors). Enhancement of tumor metastasis, as induced by hydrocortisone pretreatment, was not produced by the administrative of sex steroids (testosterone and estradiol) or of the immunosuppressant, 6-mercaptopurine. Heparin and dextran sulfate administration had little effect on the progress of tumor metastasis in hydrocortisone-treated mice. This indicated that hydrocortisone's effect on tumor metastasis was indepedent of the steps of the immune reaction or of blood coagulation. In a tracer experiment with ^{125}I-Labeled tumor cells, hydrocortisone pretreatment significantly increased the intrapulmonary retention of hypotetraploid clone 1 tumor cells from 1 to 72 hr after tumor inoculation, the time lag required for establishment of metastatic foci in the lung. Hydrocortisone pretreatment also temporarily increased tumor retention (hyperdiploid and hypertetraploid stocks) in the liver. No correlation was found between tumor cell size and the differential distribution of metastatic tumors. In an attempt to use this system for chemotherapeutic study, cyclophosphamide administration caused a significant prolongation of survival time, and often a complete prevention of tumor metastasis in hydrocortisone-conditioned mice. This system may serve as a simulation model for the study of metastasis prevention.

0410 GASTRIC NEOPLASMS IN THE DOG: A REPORT OF 20 CASES. (Eng.) Sautter, J. H. (Coll. Vet. Med., Univ. Minnesota, St. Paul); Hanlon, G. F. *J. Am. Vet. Med. Assoc.* 166(7):691-696; 1975.

Reports of 20 cases of gastric neoplasms occurring in dogs in the north central United States were documented. Seventeen of the malignant tumors occupied the lower two-thirds of the stomach; three were located in the lesser curvature. Morphological examination revealed that, of the 14 carcinomas, nine varied considerably in the amount of associated fibrous tissue (scirrhous type); metastasis appeared to be a function of the duration and degree of malignancy. The neoplasms were categorized as carcinoma (five), adenocarcinoma (two), scirrhous carcinoma (five), and scirrhous adenocarcinoma (five). In addition, three lymphosarcomas, two leiomyomas, and one leiomyosarcoma were found. The mean age of the dogs was 9.7 yr, with a male: female ratio of 12:8, and no breed preference. Consistent clinical signs included vomiting, anorexia, and weight loss, with occasional occurrence of anemia, diarrhea, melena, and hematemesis. All dogs in the study subsequently died or were euthanized. Radiographic features observed in two of six dogs radiographed consistently revealed a thickening and rigidity of the gastric wall, distortion of the gastric lumen and derangement of the rugal folds, filling defects, and a marked delay in gastric emptying time. In contrast to various observations made on the incidence of gastric neoplasia in man, no implicating factors were yet associated with the dogs.

0411 PRIMARY GRANULOCYTIC SARCOMA OF THE SMALL BOWEL. (Eng.) Brugo, E. A. (Univ. Texas Med. Branch, Galveston); Larkin, E.; Molina-Escobar, J.; Costanzi, J. *Cancer* 35(5):1333-1340; 1975.

A case report of a retrospective analysis of primary granulocytic sarcoma, and a brief literature review on granular sarcomas were presented. A 38 yr-old male Caucasian initially presented with partial small bowel obstruction. A partial resection of the proximal ileum revealed a moderately firm, nonulcerating 6 x 4 x 4-cm tumor. Microscopic sections and electron microscopy studies were reported as an undifferentiated malignant tumor, suggesting a reticulm cell sarcoma. Subsequent hospital admittances were due, sequentially, to right leg pain and atrophy of the right quadriceps muscle, epigastric pain revealing polypoid lesions at gastroscopy, severe abdominal pain diagnosed as reticulum cell sarcoma, repeated abdominal pain, and rectal bleeding. At this sixth admission, there was obvious sarcomatous infiltration of the left eyelid, bone marrow aspiration suggesting a malignant histiocytosis, and persistent anemia; death followed within six days. The autopsy reported extensive neoplastic involvement of the mediastinal and mesenteric lymph nodes, diffuse invasion and bone marrow replacement, and diffuse meningeal spread. Of particular significance was the presence of a primary granulocytic sarcoma three yr prior to the acute granulocytic leukemia, yet diagnosed only retrospectively. Review of the bone marrow aspiration performed before death revealed a hypercellular marrow with few plasma cells, large prominent cells with phagocytic inclusions, and large nucleoli. Paraffin sections of the original tumor showed primitive cells with overlapping nuclei, while electron microscopy demonstrated cells with different stages of granular development. A brief literature review focused on the gross features of solid myeloid tumors and chloromas, followed by a discussion of factors contributing to a mistaken diagnosis of histiocytic lymphoma. Characteristics of the tumor particularly noted were its location, possible involvement or compression of nerves resulting in mus-

cular atrophy after one yr, and its proceding leukemia by slightly more than one yr.

0412 BENIGN SMALL BOWEL TUMOR. (Eng.) Wilson, J. M. (Cornell Univ. Med. Cent., New York, N. Y.); Melvin, D. B.; Gray, G.; Thorbjarnarson, B. *Ann. Surg.* 181(2):247-250; 1975.

The clinical records and histological sections of 84 cases (36 males and 48 females) of benign bowel patients of the New York Hospital - Cornell Medical Center from 1932-1972 were presented. Most of the patients were 50 to 60 yr old. Clinical presentation, pathologic findings, management and results were tabulated and compared to the collected published experience of about 2000 cases. Manifestations of systemic disease (e.g., Peutz-Jegher's syndrome and Von Recklinhausen's disease), congenital anomalies, periampullary tumors, and ileocecal valve tumors were excluded from the study. Six types of benign tumors were found: 36 leiomyomas, 22 lipomas, nine angiomas, six neurofibromas, four fibromas and seven adenomas. Seventy-eight patients were sufficiently symptomatic to require operation and six were diagnosed by autopsy. The most common symptom was obstruction (42% of the cases), followed by hemorrhage (34%) and pain (22%). The relative frequency varied with tumor type. Because of the nonspecificity of the signs and symptoms and difficulty with x-ray diagnosis, the possible existence of small bowel cancer often may only be established on the operating table.

0413 CARCINOEMBRYONIC ANTIGEN (CEA) LEVELS AND TUMOR HISTOLOGY IN COLON CANCER. (Eng.) Bivins, B. A. (Univ. Kentucky Med. Cent., Lexington); Meeker, W. R., Jr.; Griffen, W. O., Jr. *J. Surg. Res.* 18(3):257-261; 1975.

The relationship of tumor histology to carcinoembryonic antigen (CEA) titer, was studied in human colonic carcinoma. Forty-two patients with adenocarcinoma of the colon had plasma CEA determined preoperatively, and operative specimens were taken for detailed study of the primary tumor and lymph nodes. Nine of the patients had CEA levels below the normal (2.5 ng/ml), whereas 33 patients had a CEA titer above this level. CEA level did not correlate with Duke Stage evaluation of the tumor, grade of differentiation, or level of mucous production. In contrast, tumor necrosis and vascular invasion were significantly correlated with CEA levels above 2.5 ng/ml. Of the 33 patients with high CEA levels, 28 had vascular invasion and 17 had necrosis. There was no significant correlation with lymph node metastases, lymphoplasmocytic infiltrates, or nodal reactions. It is concluded that elevated CEA levels can be correlated with vascular invasion and necrosis of colonic adenocarcinomas.

0414 GARNER'S SYNDROME AND FIBROMATOSIS: REVIEW OF THE PROBLEM AND REPORT OF A CASE. (Eng.) Shons, A. R. (Univ. Minnesota Hosp., Minneapolis); Estrin, J.; Najarian, J. S. *Dis. Colon Rectum* 18(2): 128-133; 1975.

A case report of a 30 yr-old man illustrates the aggressive potential of benign, soft-tissue processes of Gardner's syndrome, a disease characterized by intestinal polyposis, bony exostoses and multiple soft-tissue abnormalities. In 1969, the subject underwent subtotal colectomy and ileosigmoidostomy for multiple benign colonic polyps. Diagnosis of the syndrome was then made on the basis of a dominant inheritance pattern in seven members of two generations of the maternal side of the family. In 1971, exploratory laparotomy and biopsy revealed a benign mesenteric fibroma. In 1974, pain in the upper quadrant and left shoulder, fever, and leukocytosis developed. An upper gastrointestinal series revealed displacement of the stomach, small bowel, and duodenum. Angiography demonstrated the neovascularity of the tumor lying anteriorly over the proximal superior mesenteric artery. The fourth portion of the duodenum and all but a single loop of midjejunum of the small intestine were tightly encased in the tumor mass. The tumor, a benign fibroma (weight, 7 lbs), was excised. No postoperative complications were apparent ten months later. The authors believe that the long-term outlook for patients with a firbromatous variant of Gardner's syndrome is not favorable. Life-threatening components are the malignant behavior of histologically benign intra-abdominal fibromatous and the malignant potential of colonic polyposis. Small tumors may be completely removed, but the operation may stimulate the desmoid process throughout the abdomen resulting in death from intestinal obstruction or perforation.

0415 GEMISTOCYTIC ASTROCYTES IN GLIOMAS: AN AUTORADIOGRAPHIC STUDY. (Eng.) Hoshino, T. (Sch. Med., Univ. California, San Francisco); Wilson, C. B.; Ellis, W. G. *J. Neuropathol. Exp. Neurol.* 34(3):263-281; 1975.

The biological activity (i.e., rate of cell division, cellular origin, and relation to tumor anaplasm and degeneration) of gemistocytes and giant neoplasmic astrocytes was studied as part of an overall examination of cell kinetics in human gliomas. Tritiated thymidine (10 mCi dose) was administered i.v. or intracarotidly immediately before craniotomy in seven patients with malignant cerebral gliomas and the uptake by multiple cell types was compared at varying time intervals. Autoradiographs of biopsy specimens (excised 2-4 hr after injection) and autopsy specimens (obtained three weeks to six months after injection) from three of these patients revealed no gemistocytic astrocytes and only a few giant (astrocytes, despite a high overall labeling index of 5-10%. Scattered foci of labeled gemistocytes in autopsy specimens were also apparent, despite a sharp drop in overall labeling index. In histologic sections of these specimens, gemistocytes and giant cells occurred as the major cell types on a irradiated tumor, in large clusters near foci of degeneration and as isolated cells in anaplastic foci. This suggests that: (1) gemistocytes and giant astrocytes are similar in origin and growth potential regardless of minor variations in morphology; (2) they multiply slowly, if at all, and (3) are closely related to regressive changes within the tumor. These two cells types may reflect profound proliferative activity in adjacent cells. In addition, the labeling index and

malignant potential of the tumor as a whole depends upon the more rapidly dividing tumor elements. Thus, if gemistocytes and giant cells do indicate malignancy, it is secondarily. The biologically harmless gemistocyte may be the loser in an intense competition for substrates needed in cell proliferation.

0416 MALIGNANT SOFT TISSUE TUMORS OF PROBABLE HISTIOCYTIC ORIGIN (MALIGNANT FIBROUS HISTIOCYTOMAS): GENERAL CONSIDERATIONS AND ELECTRON MICROSCOPIC AND TISSUE CULTURE STUDIES. (Eng.) Fu, Y. S. (Coll. Physicians Surg., Columbia Univ., New York, N. Y.); Gabbiani, G.; Kay, G. I.; Lattes, R. *Cancer* 35(1):176-198, 1975.

Four cases of malignant fibrous histiocytoma were studied by correlated light and electron microscopy. The fine structural description of these lesions was clarified and the value of electron microscopy as a diagnostic and/or prognostic aid in the evaluation of unusual soft tissue neoplasms was explored. Tissues for light and electron microscopic examination were fixed in Bouin's solution and processed by routine procedures. Thick sections (1-2 μ) were stained with toluidine blue or Paragon Triple stain. The thin sections used for electron microscopy were mounted on formvar-coated or uncoated copper grids, stained with uranyl acetate and loed citrate, and carbon-coated. For tissue culture, tumor fragments from one case were explanted into chicken plasma clot and fed with nutrient medium containing human placental serum, beef serum ultrafiltrate, and chicken embryo extract (9-day, 50%). The Maximow-cover slip hanging-drop method was employed. Gluteraldehyde fixation of the explant was done at the end of 20 days. The results of light and electron microscopic study confirmed the presence of histiocyte-like and fibroblast-like cells in histiocytomas. An undifferentiated cell type, giant cells, xanthomatous cells, and rare cells with morphological characteristics intermediate between those of histiocytes and fibroblasts were also seen. Nuclear body-type inclusions were commonly seen in both principal cell types in all four cases, as were the somewhat less common cytoplasmic inclusion bodies. Electron microscopic study demonstrated only fibroblast like, histiocyte-like, and xanthomatous cells. It is suggested that both principal cell types in this tumor may derive from the same undifferentiated stem cell.

0417 HEMANGIOMATOSIS OF THE SKIN AND GASTROINTESTINAL TRACT: REPORT OF A CASE. (Eng.) Hagood, M. F. (Alton Ochsner Med. Found. Clin., New Orleans, La.); Gathright, J. B., Jr. *Dis. Colon Rectum* 18(2):141-146; 1975.

A case report of a 34 year old Caucasian woman with hemangiomatosis of the skin and gastrointestinal tract was reported, including diagnosis and treatment. The patient had a bloody diarrhea of one year's duration. Past medical history was unremarkable except for a small blue lesion which developed on patient's scalp at age 22. Similar lesions were noted on the face and back. Physical examination showed a raised, bluish rubbery-feeling lesion on scalp and temple. There were also numerous bluish, s.c. lesions over the lumbar area. Other tests performed were: proctoscopy, upper gastrointestinal series, biopsy of skin and rectal lesions, barium enema, colonoscopy, and gastroscopy. They revealed numerous raised, submucosal polypoid lesions in duodenum, upper jejunum, sigmoid rectum, and descending colon. The esophagus and upper stomach were normal. Selective abdominal angiography confirmed multiple hemangiomas involving the liver, duodenum, proximal jejunum, descending and sigmoid colon, and rectum. Hemoglobin was 11.5 g/100 ml and hematocrit was 34%. Bleeding and clotting time were normal. Due to extensive involvement of the gastrointestinal tract and the lack of massive bleeding, surgery was not indicated. Control of the symptoms with anticholinergics for diarrhea and p.o. iron therapy for anemia was elected. The difficulty of diagnosing hemangiomas is discussed; the presence of cutaneous "blue-rubber bleb nevi" is concluded to be an important diagnostic tool.

0418 THE ULTRASTRUCTURE OF THE ABNORMAL RETICULUM CELLS IN HODGKIN'S DISEASE. (Eng.) Carr, I. (Dep. Pathol., Univ. Sheffield, England). *J. Pathol.* 115(1):45-50; 1975.

The ultrastructure and derivation of abnormal reticulum cells of Hodgkin's disease and the relation to adjacent cells were studied with electron microscopy. Lymph node tissue from 23 patients with Hodgkin's disease, and spleen tissue from four of these patients, were examined and compared with lymphoreticular tissue of six control patients without malignant lymphoma. The abnormal reticulum cells were large (10/25 μm) with poorly electron dense cytoplasm which contained a cluster of organelles often related to nuclear indentation. The nucleus was large, with a prominent nucleolus, elongated mitochondria (< 3 μm) usually clustered near the nucleus and around the cell center, and a conventional Golgi apparatus and centriole. The cell margin usually showed cytoplasmic processes. The cells were seen singly or in pairs, surrounded by rings of lymphocytes and, less often, macrophage processes. These features of the atypical neoplastic reticulum cell suggest macrophage lineage and may suggest a lymphoreticular reaction to the neoplastic reticular cells. The fibrosis may also represent a host reaction.

0419 THYMUS-DEPENDENT LYMPHOCYTE LEVELS IN BRONCHOGENIC CARCINOMA: CORRELATIONS WITH HISTOLOGY, CLINICAL STAGE, AND CLINICAL COURSE AFTER SURGICAL TREATMENT. (Eng.) Dellon, A. L. (Cancer Inst., Bethesda, Md.); Potvin, C.; Chretien, P. B. *Cancer* 35(3):687-694; 1975.

Cellular immunologic competence in 112 patients (mean age, 58 yr) with bronchogenic carcinoma was determined by the spontaneous lymphocyte rosette (T cell) assay in preoperative patients, sequentially in patients after surgical resection and in cured patients. T cell levels were compared to 237 controls (healthy volunteers, 20-80 yr old). T cell levels of preoperative patients with localized tumors were significantly

lower than controls. The levels declined progressively with advanced stages of the disease among patients with squamous cell, oat cell and undifferentiated carcinomas, but not among patients with adenocarcinoma. Squamous carcinoma patients considered cured had persisting low T cell levels. Cured adenocarcinoma patients had normal levels. Serial determinations showed that a fall in T cell levels preceded the development of clinically evident metastases by an average of 2.5 months. Postoperative patients with rising T cell levels remained clinically free of the disease. The results indicated that T cell levels correlated with the extent of the tumor and clinical course of patients. This assay may provide a rational basis for the selection of patients who are a high risk for recurrence after surgical resection and of those who may benefit from early institution of adjunctive therapy.

0420 INTRANUCLEAR INCLUSIONS IN BENCE JONES LAMBDA PLASMA CELL MYELOMA. (Eng.) Cohen, H. J. (Duke Univ. Med. Cent., Durham, N. C.); Lefer, L. G. *Blood* 45(1):131-139; 1975.

A 54-yr-old male with plasma cell myeloma producing only Bence-Jones lambda protein was found to have pale intranuclear inclusions in the majority of the bone marrow plasma cells. Light and electron microscopy and histochemical studies revealed that these inclusions were non-electron dense; they were composed of a proteinaceous material bound by a single well-defined membrane which contained no cytoplasmic structures and they were PAS-negative. No intracytoplasmic inclusions were present and there was no indication of perinuclear cistern dilation. Thus, the inclusions may represent intranuclear protein synthesis with anomalous release in these abnormal cells.

0421 THE METASTATIC SPREAD OF MYELOMA AND LEUKEMIAS IN MEN. (Eng.) Bross, I. D. J. (Roswell Park Mem. Inst., Buffalo, N. Y.); Viadana, E.; Pickren, J. W. *Virchows Arch* [*Pathol Anat*] 365(2):91-101; 1975.

Five hundred and eighty autopsy records on patients with plasma cell myeloma or leukemia were used to elucidate the metastatic processes in solid tumors, sometimes referred to as "cascade analysis" (diffusion of cancer in a stepwise manner; from primary to generalized metastasis). Each autopsy report included age at time of diagnosis, sex, diagnosis by site and histology, approximate time of diagnosis and a detailed description of the presence or absence of metastases at 48 sites. Cascade analysis was employed to determine the events connecting the primary site with generalized metastatic disease, the sequence of the sites and the role of the sites in the overall process. The method involves (1) production of the pertinent 2 x 2 tables for the occurrences of metastases at different sites, (2) determination of the direction of seeding by using the Sign test for the cells where one site is positive but the other is not, and (3) determination of the extent of the effect by a chi-square on the 2 x 2 table. The spleen and liver were the two major organs affected in the metastatic spread of myeloma and leukemia. The majority of the sign tests were significant at a 5% probablity level, the route of spread being from the spleen to the lungs *via* the liver. Detailed explanation of the operative mechanism of the cascade analysis of metastases was not resolved in this paper. However, it does present some hypothetical resolutions concerning the mechanism of metastasis.

0422 BIOLOGICAL BEHAVIOR OF MALIGNANT MELANOMA CELLS CORRELATED TO THEIR SURVIVAL *IN VIVO*. (Eng.) Fidler, I. J. (Sch. Dent. Med., Univ. Pennsylvania, Philadelphia). *Cancer Res.* 35(1):218-224; 1975.

C57BL/6 mice were injected i.v. with B16 melanoma tumor cells to determine the tumor cell characteristics which influence experimental metastasis. The secondary tumors were collected two to three weeks later, placed in tissue culture and injected into new mice. The number of lung tumor nodules increased with each successive passage. The incidence of spontaneous pulmonary metastasis following s.c. injection of tumor cells was found to be higher in the F_{11} melanoma line than in the F_5 and F_1 lines. The tumor cell lines were labeled *in vitro* with (^{125}I)-5-iodo-2^1-deoxyuridine, injected i.v. into normal mice, and the lungs and blood were processed and monitored for radioactivity. Retention of viable tumor cells in the lungs was greatest for the cells which produced the highest incidence of metastasis. *In vitro* tumor cell-lymphocyte clumping was determined using normal lymphocytes, and lymphocytes from mice immunized against the B16 melanoma. The degree of clumping was greater for the B16-immunized lymphocytes than for the normal lymphocytes, and appeared to be greater in the F_{10} line than in the F_1 melanoma line. The results indicate that an increased retention of malignant cells in a tissue capillary bed may increase the chances for metastatic development. It is suggested that circulating malignant cells may possess unique qualities which enhance their chances for survival.

0423 ELECTRON MICROSCOPE DIAGNOSIS OF MEDULLOEPITHELIOMA. (Eng.) Jakobiec, F. A. (Coll. Physicians Surg., Columbia Univ., New York, N. Y.); Howard, G. M.; Ellsworth, R. M.; Rosen, M. *Am. J. Ophthalmol.* 79(2):321-329; 1975.

Electron microscopy was used as an aid in the diagnosis of medulloepithelioma of the eye in a 20-month-old boy suffering from two white cysts floating in the anterior chamber of the left eye. An operation was performed in which two gelatinous cysts (1 to 2 mm in diameter) were removed from the area of infection. A tissue section from one cyst was fixed in glutaraldehyde. Ultrastructural findings implicated the diagnosis of medulloepithelioma. Tumor cells (forming lumina and displaying neuronal type cilia), neurotubules and a complicated band of apical desmosomal junctions were found in each flocculus. This finding was significant in differentiating medulloepithelioma from retinoblastoma. It is sug-

gested that electron microscopy as a means of diagnosis allows for a trial period of conservative cryotherapy directed at the tumor and the associated glaucoma.

0424 ERYTHROPLAKIA OF THE ORAL CAVITY. (Eng.) Shafer, W. G. (Indiana Univ. Sch. Dentistry, Indianapolis, Indiana 46202); Waldron, C. A. *Cancer* 36(3): 1021-1028; 1975.

A series of cases of oral erythroplakia are reported, in view of the general recognition that it is apparently a premalignant disease. A series of 58 cases of oral erythroplakia was retrieved from 65,354 consecutively accessioned biopsy-surgical specimens. The disease was found to have no apparent sex predilection (31 males and 27 females) and was most frequently seen during the sixth and seventh decades. The most common site of occurrence in females was the mandibular alveolar mucosa-mandibular gingiva-mandibular sulcus, whereas this was the least common site in males. The floor of the mouth was the most common site in males, followed by the retromolar area in both males and females. The histologic findings emphasized the serious nature of the disease since 91% of the specimens were either invasive carcinoma, carcinoma *in situ*, or severe epithelial dysplasia.

0425 PRIMARY OSTEOGENIC SARCOMA OF THE KIDNEY. (Eng.) Chambers, A. (Middletown Hosp., Ohio); Carson, R. *Br. J. Radiol.* 48(568):316-317; 1975.

The case history of a 45-yr-old man with metastatic osteogenic sarcoma of the kidney was presented. The patient reported an episode of left flank pain. Upon examination two months later, a left abdominal mass was found. Laboratory studies and bone marrow surveys were normal. The urine contained a few RBCs and WBCs. A urogram showed no excretion on the left. A radical operation was performed with removal of the left kidney; part of the colon adherent to the kidney and a liver wedge were resected. The patient's condition deteriorated over the next nine months and he died with multiple pulmonary metastasis, hepatic metastasis, and local recurrence. The primary neoplasm in the left kidney showed distinct differentiations to mature bone. Immature elements of mesenchymal origin were present in the perinephric fat, but there were sharply localized nodules of sclerotic bone in this area also. Osteogenesis was present in all metastic nodules in the lung, liver, and cranial dura.

0426 A HUMAN MALIGNANT CELL LINE ESTABLISHED FROM ASCITES OF PATIENT WITH EMBRYONAL CARCINOMA OF OVARIUM. (Eng.) Kimoto, T. (Kawasaki Med. Univ., Okayama, Japan); Ueki, A.; Neshitani, K. *Acta. Pathol. Jpn.* 25(1):80-98; 1975.

The establishment of a malignant tumor cell line from the ascites of a 63 yr-old female with primary ovarian tumor (embryonal carcinoma) was described. Autopsy specimens revealed cancerous ascites due to cancer metastasis to the peritoneum, histological pictures of metastatic lesion resembling cystadenocarcinoma, and the differentiation of cells to papillary tubular and reticular, non-epithelial cells. The cells obtained at autopsy from the peritoneum were washed, centrifuged, then cultured with McCoy 5 A or RPMI 1640. The cells derived from ascites were mesothelioma-like. Three week cultures revealed slender cellular projections which connected some of the cells, and occasional fibrous projections of relatively wide, strongly basophilic cytoplasm, or large spindle fibroblast-like cells with strongly basophilic anaplasia. In contrast to normal cells, a mosaic colony was formed, contact inhibition was lost, and colonies of epithelioid, spindle-shaped mesothelial, or fibroblastic tumor cells were found. Polynuclear giant cells with a rosette nucleus and many mitotic figures were also found. Electron microscopy revealed somewhat deformed mitochondria with well-developed endoplasmic reticulum; the tumor cells were found to engulf exogenous peroxidase and to phagocytize chondroitin sulfate colloid iron. The cell line proliferated characteristically well, reflected the inherent properties of the original tumor, and had a chromosome number of about 50 in its 95th passage, 19 months after the start of subculture. This cell line differed from *in vitro* SV40- transformed cells. It appeared to be derived from ovarian tumor cells that had suspended and proliferated in ascites.

0427 EPITHELIAL AND MUSCLE ANTIGENS IN BENIGN CYSTIC TERATOMAS OF THE OVARY. (Eng.) Pertschuk, L. P. (Dep. Pathol., State Univ. New York, Brooklyn). *Cancer Res.* 35(3):750-753; 1975.

Some of the antigenic properties of benign ovarian cystic teratomas (dermoid cysts), when reacted with specific human antisera in a defined immunofluorescence system, were examined. To study tissue taken from the mamilla of 30 teratomas, specific human antisera obtained from patients with various autoimmune disorders were used for squamous epithelial intercellular areas (pepphigus sera), squamous epithelial basement membranes (bullous pemphigoid sera), smooth muscle (chronic active hepatitis sera) and striated muscle (thymoma sera). The specificity of each serum was determined by immunofluorescence testing on a variety of substrates; normal human sera and 0.9% NaCl solution served as controls. No specific staining was apparent in any of the dermoid cysts studied with skeletal muscle antibody. Studies with intercellular substance (ICS) and substance basement membrane (BM) demonstrated that this epithelium was antigenically different from normal stratified squamous epithelium and the epithelium of benign squamous cell tumors. Using ICS as a hallmark of differentiation, the findings indicated a slow rate of regeneration for the squamous epithelium within the peculiar environment of a benign cystic tumor, allowing the basal cells more time to partially mature. No skeletal muscle was found to be involved. Whereas previous reports indicate smooth muscle presence in most cystic ovarian teratomas, this study reported it identified in 73% of the specimens. The presence of ICS, BM, and

smooth muscle antibody in the squamous epithelial and smooth muscle components supported the concept that the benign cystic ovarian teratomas are composed of mature, heterotropic elements.

0428 THE PATHOLOGIC BEHAVIOR OF PRIMARY VAGINAL CARCINOMA AND ITS RELATIONSHIP TO CERVICAL CANCER (Eng.) Murad, T. M. (Univ. Alabama Med. Cent., Birmingham); Durant, J. R.; Maddox, W. A.; Dowling, E. A. *Cancer* 35(3):787-794; 1975.

The surgical pathology files at the University of Alabama Medical Center and the University of Alabama Hospital from 1958-1973 were analyzed and the relation between primary and secondary vaginal cancer and cervical cancer discussed. Records, slides, type of therapy, histopathology of the tumor, and the length of time the patients survived from treatment were reviewed. The medical center files revealed 157 cases of vaginal carcinoma. The hospital reported 1825 cases of cervical carcinoma. Of the patients with vaginal cancer, complete records were available for only 141; of those, 37 had primary (seven with carcinoma *in situ*, 27 with invasive epidermoid carcinoma, and three with invasive adenocarcinoma) and 104 had secondary vaginal cancer. Microscopic examinations revealed that the carcinomas were frequently lateral spreading or papillary. In a few instances, the growth pattern was submucosal. Prognosis appeared to be related to the stage of the disease. Primary vaginal cancer was far less prevalent than cervical cancer, but patients treated for cervical carcinoma with irradiation showed a greater incidence of vaginal cancer. Vaginal carcinomas associated with cervical cancer clustered either within one or five yr after therapeutic treatment. It is suggested that patients treated for cervical cancer continue with follow-up examinations as they display a continued susceptibility to the development of vaginal carcinoma even after the apparent cure of the original disease.

0429 TRANSPLANTABLE METASTASIZING PROSTATE ADENOCARCINOMAS IN RATS. (Eng.) Pollard, M. (Lobund Lab., Univ. Notre Dame, Indiana); Luckert, P. H. *J. Natl. Cancer Inst.* 54(3):643-649; 1975.

Three spontaneous prostate adenocarcinomas in germfree Lobund Wistar rats were transplanted into other rats and propagated for study. The three rats were autopsied aseptically, and the tumors were minced and inoculated s.c. into the dorsolumbar region of 12-day-old and weanling Wistar, Sprague-Dawley, and Fischer rats. Cells from one tumor (designated I) were also transplanted to germfree rats. When the transplanted tumors appeared, one was minced and administered s.c. to normal weanling and older (four months to one yr of age) Wistar rats. Weanling Wistar rats from two commercial sources were also inoculated. Except for the rats killed for these passages, the remaining rats were observed for two to four months and autopsied. Five weeks after inoculation, the transplanted tumors from line I were excised from eight rats and compared with untreated controls. In 12 consecutive passages of tumor I cells in weanling rats, tumors developed in all inoculated areas, and showed no microbial contamination. The four rats with first passage tumors had small nodules within four days which were attached to the skin and/or underlying muscle. After three weeks, autopsy showed the tumors had invaded the muscles and resembled the primary tumors, consisting of sheets of large epithelial cells in a stroma of connective tissue. Mitotic figures were seen in the epithelial cells. The lungs had solid metastatic tumors ranging from small nodules to large confluent masses, histologically similar to the original tumor. Three of the four had elevated WBC counts of 19,000-36,000/mm^3. Rats inoculated i.p. developed nodules on the visceral organ surfaces, occasionally penetrating the kidneys, liver, diaphragm, and lungs. Rats with peritoneal tumors had WBC counts of 49,000-128,000/mm^3. In the eight rats from which type I tumors had been taken at five weeks, all had metastatic lung and lymph node tumors. Small tumors were seen in the lungs of other rat groups 28 days after s.c. inoculation. Wistar rats from commercial sources showed less susceptibility to tumors I and II. Sprague-Dawley and Fischer rats were refractory to all three tumors. Tumor I transplanted to germfree rats showed the same transplantation pattern as in the non-germfree rats. Tumor II was transplanted through ten series of Wistar rats; they showed less tendency to ulcerate and less necrosis, but spread to the lungs and lymph nodes. It is concluded that these carcinomas provide unique experimental model systems in which tumor histologic characteristics are retained through spread from the inoculation site to lungs and lymph nodes.

0430 SKIN CANCER IN BLACK PATIENTS. (Eng.) Fleming, I. D. (Univ. Tennessee Med. Units, Memphis); Barnawell, J. R.; Burlison, P. E.; Rankin, J. S. *Cancer* 35(3):600-605; 1975.

Clinical course and pathology of 58 cases of skin cancer in black patients were presented. Thirty-eight cases of squamous cell carcinoma were noted; it was the most common form in black patients. Sixty-one per cent of the squamous cell carcinomas developed in unexposed areas and 41% of these cases had predisposing factors such as burn scars or chronic infections. There was 29% mortality within this group. Of the 13 cases with malignant melanoma, 76% occurred on the plantar surface of the foot. Four patients were treated by surgical excision, six required amputation, and three received radiation and chemotherapy; eleven of the patients died. Six out of seven cases of basal cell carcinoma occurred in the head and neck region and were controlled by surgical excision. Of the 38 cases of squamous cell carcinoma studied, 61% were female; while in white patients, this carcinoma was seen more frequently in males. Also, 23 of these 38 cases developed in skin not chronically exposed to light, while the opposite was true in white patients. Results also showed that squamous cell carcinoma is more lethal in black patients than in white patients. In cases of malignant melanoma, 9 of 13 black patients had lesions on the foot, which is significantly different from the distribution in white

patients. It is apparent that skin cancer in black patients presents a different clinical picture than that seen in white patients, and it is important to consider these factors when planning therapy.

0431 MALIGNANT LYMPHOMA OF THE THYROID FOLLOWING IRRADIATION. (Eng.) Bisbee, A. C. (Good Samaritan Hosp., Phoenix, Ariz.); Thoeny, R. H. *Cancer* 35(5):1296-1299; 1975.

A unique case of a primary lymphocytic lymphoma of the thyroid occurring in the previously irradiated gland is reported. A Caucasian woman had received a total of 150 R irradiation for an enlarged thymus at the age of 4 1/2 months. She presented with a 2-cm nodule in the left lobe of the thyroid at age 19 upon which subtotal thyroidectomy was performed; the left lobe, isthmus and part of the right lobe were removed. Examination revealed a chronic lymphocytic thyroiditis, within which an encapsulated lesion was diagnosed as a malignant lymphoma. No other sites of involvement were noted, and a total of 5,000 rads was applied to an anterior field covering the neck, supraclavicular areas, and the superior mediastinum. The case was unique due to the relatively young age of the patient, the history of previous irradiation, and the limited occurrence of the disease. While chance occurrence may be possible, it was noted that the thyroid lymphoma had developed following a latent period similar to that reported in thyroid carcinoma in previously irradiated children.

0432 THE CYTOLOGY OF TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER. (Eng.) Kern, W. H.; (Dept. Pathology, Hosp. Good Samaritan, Los Angeles, Calif.). *Acta Cytol. (Baltimore)* 19(5):420-428; 1975.

Evaluation of qualitative and quantitative aspects of exfoliated epithelial cells was made from normal, hyperplastic, and neoplastic transitional epithelium of the urinary bladder. Urine specimens were obtained from 77 patients with transitional cell tumors of the urinary bladder, 15 patients with inflammatory lesions, and five normal individuals. One hundred cells from each case were counted and determined as normal, atypical or neoplastic. All patients with neoplastic disease had over 100 neoplastic cells. The percentage of neoplastic to atypical cells ranged from 25% for papillary carcinomas (Grade I) to 36% for poorly differentiated carcinomas. Malignant cell nuclei were more hyperchromatic than normal. Normal transitional cells were an average of 341 μ^2 and were larger than tumor cells. The nuclear area of normal cells was 36 μ^2; benign atypical cells were 52 μ^2; cells from papillary carcinoma, Grade I were 54 μ^2; papillary carcinomas, Grade II were 78 μ^2; and transitional cell carcinoma Grades III and IV were 90 μ^2. The results indicate that quantitative differences between normal transitional cells and exfoliated cells from transitional cell carcinomas exist. Cells from carcinomas (Grade I) resemble hyperplastic or atypical transitional cells that are different from normal urothelial cells.

0433 METASTASIS OF METASTASES. (Eng.) Hoover, G. C., Jr.; (Massachusetts General Hosp., Boston, Mass. 02114); ketcham, A. S. *Am. J. Surg.* 130(4):405-411; 1975.

The technique of parabiosis provided a system for studying the metastatic potential of experimental metastases. Inbred C57Bl male or C_3H female adult mice were injected with 1 x 10^6 cells of methylcholanthrene-induced sarcoma (MCA-1), Lewis T241 sarcoma, or mammary adenocarcinoma. Subsequently occurring tumors were amputated in two weeks. Two syngeneic animals were joined surgically to form a common circulatory system. The pair was examined macroscopically to quantitate pulmonary metastases. In one experiment, host mice were injected with tumor cells before parabiosis; 40-94% of the guest mice demonstrated metastasis of metastases after being joined. During another experiment, the guest mice were immunized to the primary tumor; all immune guests demonstrated metastasis to the lungs after parabiosis. In an immune challenge experiment, all of the immune guests developed pulmonary metastases, indicating that known immunity was overcome in the system. Immunizing the guest thus does not prevent formation of metastasis. This study demonstrates the spontaneous metastasis of metastases despite immunity, indicating either that metastasizing cells are resistant to immune mechanisms, or that serum-enhancing factors are present.

0434 THE MECHANISMS OF GLUTARALDEHYDE-FIXED SARCOMA 180 ASCITES CELL AGGREGATION. (Eng.) Skehan, P. (Univ. Colorado Medical Center, Denver, Colo. 80220). *J. Membr. Biol.* 24(1):87-106; 1975.

The mechanisms of cell aggregation by glutaraldehyde-fixed sarcoma 180 ascites cells were investigated using sediment height analysis. The aggregation of these cells proceeds by a polymer bridging mechanism in which the surface molecules of one cell associate directly with the surface molecules of adjacent cells by nonbonding interactions. The ability of adhesive surface macromolecules to serve as polymer bridges is regulated by hydrophobic and coulombic interactions. Hydrophobic interactions are not significantly involved in polymer bridging *per se*, but instead appear to operate either intramolecularly or between adjacent molecules of the same cell surface, and regulate the conformation and ability of such molecules to form stable intermolecular associations with the surface adhesive molecules of a nearby cell. A disruption of these intrasurface hydrophobic interactions generally promotes cell aggregation. Coulombic forces generated by the fixed charges of surface molecules inhibit aggregation; their diminution by charge neutralization promotes aggregation. It is likely that coulombic repulsive forces regulate intramolecular associations, interactions between adjacent molecules arising from the same cell surface, and interactions between macromolecules arising from different cell surfaces. The actual forces which serve to aggregate two fixed cells are not hydrophobic, but have characteristics commonly attributed to hydrogen bonding. Ion-pairing does not seem to play a role in the aggregation of fixed cells under physiological electrolyte conditions, nor does disulfide bridging.

0435 NEOPLASTIC CHANGE IN DUPLICATIONS OF THE ALIMENTARY TRACT. (Eng.) Orr, M. M. (St. Bartholomew's Hosp., London, England); Edwards, A. J. *Br. J. Surg.* 62(4):269-274; 1975.

0436 COLLOID CARCINOMA ARISING IN AN ANAL FISTULA: CASE REPORT WITH A BRIEF REVIEW OF THE LITERATURE. (Eng.) Raghavaiah, N. V. (Andhra Med. Coll., Visakhapatnam, India). *Aust. N.Z. J. Surg.* 45(1):97-99; 1975.

0437 SQUAMOUS-CELL CARCINOMA OF THE ANUS ARISING IN A GIANT CONDYLOMA ACUMINATUM: REPORT OF A CASE. (Eng.) Sturm, J. T. (St. Paul-Ramsey Hosp., Minneapolis, Minn.); Christenson, C. E.; Uecker, J. H.; Perry, J. F., Jr. *Dis. Colon Rectum* 18(2):147-151; 1975.

0438 ANORECTAL MELANOMA. (Eng.) Balthazar, E. J. (New York Med. Coll., N.Y.); Javors, B. *Am. J. Gastroenterol.* 63(1):79-83; 1975.

0439 CARCINOMA IN ISOLATED BLADDER AFTER ILEO-CONDUIT DIVERSION. (Eng.) Rege, P. R. (Veterans Adm. Hosp., Cincinnati, Ohio); Evans, A. T. *Urology* 5(5):652-653; 1975.

0440 SCANNING ELECTRON MICROSCOPY OF PRIMARY BONE TUMORS [abstract]. (Eng.) Pool, R. R. (Sch. Veterinary Medicine, Univ. California, Davis, Calif. 95616). *Lab. Invest.* 32(3):455; 1975.

0441 BENIGN AND SECOND MALIGNANT PRIMARY NEOPLASMS IN WOMEN DYING OF BREAST CANCER *VERSUS* LYMPHOMA [abstract]. (Eng.) Tomaszewski, M.-M. (Natl. Cancer Inst., Bethesda, Md. 20014); Henson, D. E.; Geelhoed, G. W. *Lab. Invest.* 32(3):458; 1975.

0442 SIGNET RING CELL CARCINOMA OF THE BREAST: THE MUCINOUS VARIANT OF INFILTRATING LOBULAR CARCINOMA [abstract]. (Eng.) Steinbrecher, J. S. (Univ. of Colorado Sch. Medicine, Denver, Colo. 80220); Silverberg, S. G. *Lab. Invest.* 32(3):435-436; 1975.

0443 THE INCIDENCE OF MULTIFOCAL DUCTAL CARCINOMA IN CANCEROUS BREAST AND BREASTS CONTRALATERAL TO CANCER: A STUDY OF WHOLE HUMAN ORGANS. [abstract]. (Eng.) Jensen, H. M. (Sch. Medicine, Univ. California, Davis, Calif. 95616); Wellings, S. R. *Lab. Invest.* 32(3):427; 1975.

0444 GYNECOLOGIC IMPLICATIONS OF BURKITT'S TUMOR. (Eng.) Halpin, T. F. (Mem. Hosp., Worcester, Mass.). *Obstet. Gynecol. Surv.* 30(6):351-358; 1975.

0445 CARDIAC RHABDOMYOMA: A CLINICO-PATHOLOGIC AND ELECTRON MICROSCOPIC STUDY [abstract]. (Eng.) Fenoglio, J. J., Jr. (Armed Forces Inst. Pathol., Washington, D.C.); Ferrans, V. J.; McAllister, H. A., Jr. *Lab. Invest.* 32(3):423; 1975.

0446 EFFECTS OF DIBUTYRYL CYCLIC AMP ON CHORIOCARCINOMA *IN VITRO* [abstract]. (Eng.) Garancis, J. C. (Medical Coll. of Wisconsin, Milwaukee, Wis. 53226); Hussa, R. O.; Story, M. T.; Pattillo, R. A. *Lab. Invest.* 32(3):446; 1975.

0447 MALIGNANT MELANOMA OF THE CHOROID IN AN 11-MONTH-OLD INFANT. (Eng.) Fledelius, H. (Eye Pathol. Inst., Univ. Copenhagen, Denmark); Land, A. M. *Acta Opthalmol. (Kbh.)* 53(2):160-166; 1975.

0448 ULTRASTRUCTURAL ABNORMALITIES OF MARMOSET COLON CANCER CELLS. (Eng.) Swartzendruber, D. C. (Med. Div., Oak Ridge Assoc. Univ., Tenn.); Buckland, C. H.; Lushbaugh, C. C.; Humason, G. L.; Gengozian, N. *Fed. Proc.* 34(3):825; 1975.

0449 DIFFERENTIATION OF CLONAL LINES OF TERATOCARCINOMA CELLS: FORMATION OF EMBRYOID BODIES *IN VITRO*. (Eng.) Martin, G. R. (Dept. Anat. Embryol., Univ. Coll. London, England); Evans, M. J. *Proc. Natl. Acad. Sci. USA* 72(4):1441-1445; 1975.

0450 MORPHOLOGICAL AND CYTOCHEMICAL STUDIES ON INDUCED NEOPLASMS OF COLON. (Eng.) Toth, B. (Eppley Inst. Res. Cancer, Univ. Nebraska, Omaha); Malick, L. *Fed. Proc.* 34(3):827; 1975.

0451 PRIMARY ADENOID CYSTIC CARCINOMA OF THE ESOPHAGUS: REPORT OF A CASE AND REVIEW OF THE LITERATURE. (Eng.) Pourzand, A. (Washington Hosp. Cent., D.C.); Freant, L.; Levin, R.; Peabody, J.; Absolon, K. *J. Thorac. Cardiovasc. Surg.* 69(5):785-789; 1975.

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0455 INFANTILE FIBROSARCOMA [abstract]. (Eng.) Chung, E. B. (Howard Univ. Coll. Med., Washington, D.C.); Enzinger, F. M. *Lab. Invest.* 32(3):420; 1975.

0456 GARDNER'S SYNDROME AND VILLOUS ADENOMA OF JEJUNUM. (Eng.) Grosberg, S. J. (Veterans Adm. Hosp., Brooklyn, N.Y.). *Am. Surg.* 41(3):177-178; 1975.

0457 MALIGNANT TUMORS ASSOCIATED WITH GRANULOMATOUS ENTEROCOLITIS. (Eng.) Kim, U. (Mt. Sinai Sch. Med. City Univ. New York, N.Y.); Aufses, A. H., Jr.; Kreel, I. *Am. J. Gastroenterol.* 63(1):66-70; 1975.

0458 KAPOSI'S SARCOMA OF THE CONJUNCTIVA. (Eng.) Howard, G. M. (Columbia-Presbyt. Med. Cent., New York, N.Y.); Jakobiec, F. A.; DeVoe, A. G. *Am. J. Ophthalmol.* 79(3):420-423; 1975.

0459 RENAL ADENOCARCINOMAS INDUCED BY N-4-4'-FLUOROBIPHENYL ACETAMIDE AND THEIR COMPARISON WITH HUMAN RENAL ADENOCARCINOMAS [abstract]. (Eng.) Trump, B. F. (Univ. Maryland Sch. Medicine, Baltimore, Md. 21201); Hinton, D. E.; Dees, J. H.; Heatfield, B. M.; Barrett, L. *Lab. Invest.* 32(3):438; 1975.

0460 BENIGN GRANULAR CELL TUMORS OF THE LARYNX: A REVIEW OF 36 CASES [abstract]. (Eng.) Compagno, J. (Armed Forces Inst. Pathol., Washington, D.C.); Hyams, V. *Lab. Invest.* 32(3):421; 1975.

0461 FINE STRUCTURE OF PRENEOPLASTIC CELLS IN THE LIVER [abstract]. (Eng.) Zaki, F. G. (Squibb Inst. Medical Res., New Brunswick, N.J. 08903); Greenlee, J. A.; Keysser, C. H. *Lab. Invest.* 32(3):441-442; 1975.

0462 AN EVALUATION OF THE USE OF THE SMEAR PREPARATION ON THE HEPATIC NODULE IN LIVER CARCINOGENESIS [abstract]. (Eng.) Richardson, H. L. (Office Pesticides Programs, Environmental Protection Agency, Washington, D.C. 20460). *Lab. Invest.* 32(3):432; 1975.

0463 EXPERIMENTAL STUDY ON THE DEVELOPMENT OF LIVER CELL CARCINOMA. ROLE OF HISTAMINE IN CHRONIC LIVER INJURY. (Eng.) Ishii, K. (Sch. Med., Keio Univ., Tokyo, Japan); Suzuki, O.; Kiryu, Y.; Tsuchiya, M. *Keio J. Med.* 24(1):39-47; 1975.

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0467 A NEW FUNCTIONAL CELL LINE FROM HUMAN PULMONARY SMALL CELL ANAPLASTIC CARCINOMA. II. MORPHOLOGICAL CHARACTERISTICS. (Eng.) Faulkner, C. S. (Dartmouth Med. Sch., Hanover, N.H.); Pettengill, O. S.; Maurer, L. H.; Sorenson, G. D. *Fed. Proc.* 34(3):840; 1975.

0468 CELL-SURFACE STRUCTURE IN HAIRY-CELL LEUKAEMIA. (Eng.) Katayama, I. (Univ. Massachusetts Medical Center, 55 Lake Ave. North, Worcester, Mass. 01605); Schneider, G. B. *Lancet* 1(7912):920-921; 1975.

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0472 SPONTANEOUS MALIGNANT LYMPHOMA IN NONHUMAN PRIMATES: A MORPHOLOGIC STUDY OF 43 CASES [abstract]. (Eng.) Terrell, T. G. (California Primate Res. Center, Davis, Calif. 95616); Gribble, D. H.; Osburn, B. I. *Lab. Invest.* 32(3):437; 1975.

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0475 MULTIPLE PRIMARY TUMORS: FOUR DISTINCT HEAD AND NECK TUMORS. (Eng.) Iannaccone, P. (Coll. of Physicians & Surgeons, 630 W. 168th St., New York, N.Y. 10032). *Arch. Pathol.* 99(5):270-272; 1975.

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0477 ENDODERMAL SINUS TUMOR OF THE OVARY: AN ULTRASTRUCTURAL STUDY [abstract]. (Eng.) Silverberg, S. G. (Univ. of Colorado Medical Center, Denver, Colo. 80220); Eloustein, P. A.; Nogales, F. F., Jr. *Lab. Invest.* 32(3):456; 1975.

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0485 SECONDARY CARCINOMA OF THE SMALL INTESTINE. (Ita.) Fiumara, A. (Istituto di Anatomia e Istologia Palologica dell'Universita di Catania, Catania, Italy); Lorenzetti, L.; Macca, G. *Riv. Anat. Patol. Oncol.* 38(2):265-282; 1972-1973.

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0489 GONADOBLASTOMA IN DYSGENETIC GONADS WITH A Y CHROMOSOME. (Eng.) Mulvihill, J. J. (Natl. Cancer Inst., Bethesda, Md.); Wade, W. M.; Miller, R. W. *Lancet* 1(7911):863; 1975.

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0491 MALIGNANT TESTICULAR ANDROBLASTOMA WITH GYNECOMASTIA. (Eng.) van Overeem Hansen, G. (Nyborg Hosp., Denmark). *Dan. Med. Bull.* 22(1):33-36; 1975.

0492 MORPHOLOGY OF THE TESTES AT EARLY AND LATE STAGES OF CANCER OF THE PROSTATE. (Rus.) Khmelnitskii, O. K. (No affiliation given); Medvedev, Iu. A.; Morozov, M. A. *Arkh. Patol.* 37(2):28-34; 1975.

0493 THYROID TUMORS IN THE FISCHER RAT (F-344) [abstract]. (Eng.) Cockrell, B. Y. (Litton Bionetics, Inc., Kensington, Md.); Garner, F. M. *Lab. Invest.* 32(3):420; 1975.

0494 ULTRASTRUCTURE OF TWO CASES OF ANAPLASTIC GIANT CELL TUMOR OF THE HUMAN THYROID GLAND. (Eng.) Gaal, J. M. (Dept. Pharmacol., Univ. Toronto, Canada); Horvath, E.; Kovacs, K. *Cancer* 35(5):1273-1279; 1975.

0495 SMALL CELL MALIGNANT TUMORS OF THE THYROID: A LIGHT AND ELECTRON MICROSCOPY STUDY [abstract]. (Eng.) Cameron, R. G. (Pathol. Inst., McGill Univ., Montreal, Canada); Seemayer, T. A.; Wang, N. S.; Ahmed, M. N. *Lab. Invest.* 32(3):443; 1975.

0496 ANAPLASTIC EPITHELIOMA OF THE THYROID GLAND SIX YEARS AFTER CERVICAL IRRADIATION FOR MALIGNANT LYMPHOMA. (Fre.) Voisin, M. (Hopital Notre-Dame, 1560, rue Sherbrooke est, Montreal, Que. H2L 4K8 Canada); Tawil*, E.; LeBuis, F. F.; Bettez, P.; Somma, M. *Can. Med. Assoc. J.* 113(7):648-649, 652; 1975.

0497 HISTOPATHOLOGIC FEATURES OF VAGINAL ADENOSIS AND RELATED CHANGES IN DIETHYLSTILBESTROL-EXPOSED FEMALES [abstract]. (Eng.) Hart, W. R. (Dept. Pathol., Univ. Michigan, Ann Arbor); Townsend, D. E.; Aldrich, J. O.; Henderson, B.; Roy, M. *Lab. Invest.* 32(3):426; 1975.

0498 ATYPICAL SMOOTH MUSCLE TUMORS OF THE UTERUS: A CLINICAL AND PATHOLOGIC ANALYSIS OF 26 CASES INCLUDING LEIOMYOBLASTOMA, EPITHELIOID LEIOMYOMA, AND CLEAR CELL LEIOMYOMA [abstract]. (Eng.) Kurman, R. J. (Armed Forces Inst. Pathology, Washington, D. C. 20306); Norris, H. J. *Lab. Invest.* 32(3):429; 1975.

See also:

* (Rev): 0001, 0008, 0012, 0013, 0014, 0031, 0044, 0045, 0046, 0047, 0048, 0049, 0050, 0051, 0052, 0053, 0054
* (Chem): 0061, 0083, 0084, 0099, 0106, 0111, 0115, 0116, 0117, 0143, 0173, 0177
* (Phys): 0185, 0186, 0190, 0192
* (Viral): 0223, 0226, 0239, 0244, 0249
* (Immun): 0287, 0289, 0290, 0352, 0374
* (Epid-Biom): 0506, 0508

0499 CANCER REGISTRY DATA VERSUS MORTALITY STATISTICS. (Eng.) Staszewski, J. (No affiliation). *Recent Results Cancer Res.* 50:103-110; 1975.

Mortality statistics and cancer morbidity data from the same countries were compared and the relative merits and disadvantages of these sources for the estimation of cancer risk were evaluated. The criteria used for comparison were: completeness and reliability, comparability (i.e. with the date from other sources), availability of additional information, and cost. The results indicated that for developed countries, mortality statistics and morbidity data gave similar pictures of the geographic distribution of cancer. This was true not only for the cancers which are usually fatal (e.g. lung and stomach), but also for the more curable ones (e.g. uterus and breast). Variation in the quality of cancer registration was observed; Scotland was an example of less complete registration. In a few studies, data on skin cancer was not reported to be very reliable. In spite of their differences it was concluded that information from mortality statistics and morbidity registration was complementary rather than contradictory, in that conclusions based on these two sources often coincided.

0500 THE PERSISTANCE OF DIFFERENCES IN CANCER INCIDENCE AT VARIOUS ANATOMICAL SITES 1300 YEARS AFTER IMMIGRATION. (Eng.) Jussawalla, D. J. (No affiliation). *Recent Results Cancer Res.* 50:170-181; 1975.

The magnitude and nature of the variations in the frequencies of cancers at different body sites observed among the Parsis residents of Greater Bombay, India were studied. Sites less commonly affected by cancer in the Parsis than in total Bombay population included: buccal cavity, pharynx, larynx, esophagus, and cervix. Parsis rates were higher for a number of body sites: pancreas, prostate, bladder, nervous system, female breast, and body of the uterus and ovary. Leukemia rates were also higher. Speculative explanations for the low rates of oral and pharyngeal cancers are related to the Parsis' abstinence from chewing tobacco and moderate smoking habits. It is also postualted that the high rate of breast cancer is due to other social factors: late marriage, infrequent breast feeding, and low fertility rates. The high incidence of prostate cancer may be explained by the fact that a greater proportion of the Parsis are elderly (cancer of the prostate is age-related). It is thus concluded that the differences in frequency among the various cancer sites are due to variations observed in the Parsis' habits, customs, and economic status. Further study is recommended to relate etiological factors to cancer risk at certain sites in the body.

0501 CANCER OF ASIANS IN KENYA. (Eng.) Chopra, S. A. (H. H. Aga Khan Platinum Jubilee Hosp., Nairobi, Kenya); Linsell, C. A.; Peers, F. G.; Chopra, F. S. *Int. J. Cancer* 15(4):684-693; 1975.

The frequency of various cancers among Indian immigrants in Kenya was compared to corresponding data among native Africans and Indians in the regions of India from which most of the studied population originally migrated. Data was obtained from the records of three hospitals. Patients were admitted to the study based on age, date of diagnosis, clinical history, radiological surgical evidence and religion. Asians in Kenya had an increased number of tongue, nasopharynx, esophagus, liver, breast, and eye tumors. Lymphosarcoma was more frequent in the Kenyan African than the Asian immigrant. Tumors of the mouth, oro-hypopharynx, stomach, large intestine and rectum, larynx, corpus uteri and melanoma were higher in Kenyan Asian males and were decreased in their African counterparts. No observable differences between Kenyan Asians and native Africans was noted in cancer of the cervix, lung and the development of leukemia. The data presented indicates the need for further registration and more detailed epidemiological investigations.

0502 EPIDEMIOLOGIC INVESTIGATIONS OF POSSIBLE BIOLOGICAL INTERACTIONS OF ALCOHOL AND CANCER OF THE HEAD AND NECK. (Eng.) Kissin, B. (Downstate Med. Cent., State Univ. New York, Brooklyn, N.Y.). *Ann. N.Y. Acad. Sci.* 252:374-377; 1975.

A preliminary epidemiologic study testing several specific hypotheses, exploring methodology, and indicating general trends in the clinical association of heavy alcohol ingestion and cancer of the head and neck was presented. In attempting to improve the quality of retrospective studies through the development of a more quantitative instrument, a carefully designed interview was employed. Investigations of the hypothesis that patients with head and neck cancer show a higher incidence of heavy drinking, heavy smoking, and malnutrition than other control groups yielded general support of all conclusions; however, no significant difference in the eating pattern was noted. Likewise, patients with head and neck cancers appeared to have adopted a heavier drinking and smoking pattern earlier in life, which was then maintained for a longer period of time. In relating more directly the smoking/drinking ratio to the specific sites of contact, and the concepts of an "inhalation" tract *vs* an "ingestion" tract, the data supported the specific hypothesis that the site of cancer development is related to the directness of contact with either alcohol or tobacco smoke. As a corollary to that hypothesis, it was likewise determined that even greater effects were due to hard whiskey, with its greater content of higher alcohols. Although most preliminary findings did not reach statistical levels of significance, the data suggested the existence of certain pathogenetic mechanisms through which the heavy chronic ingestion of alcoholic beverages may contribute to the development of cancer of the head and neck.

0503 ASSOCIATION OF RACE, AGE, MENOPAUSAL STATUS, AND CERUMEN TYPE WITH BREAST FLUID SECRETION IN NONLACTATING WOMEN, AS DETERMINED BY NIPPLE ASPIRATION. (Eng.) Petrakis, N. L. (G. W. Hooper Found., Dep. Intern. Health, Univ. California, San Francisco); Mason, L.; Lee, R.; Sugimoto, B.; Pawson, S.; Catchpool, F. *J. Natl. Cancer Inst.* 54 (4):829-834; 1975.

Biologic and physiologic factors associated with the availability of breast secretions by a nipple aspiration technique was investigated in 606 normal, nonlactating women. Data was obtained on race, age, menopausal status, menstrual history, use of contraceptive pills, and, for Japanese and Chinese women, cerumen type. A suction cup device was placed over the nipple and a vacuum was developed with the use of a syringe attached to the suction with plastic tubing. Preceding aspiration the surface of each nipple was cleansed of desquamated epithelium with ceruminex. Breast fluid was obtained from 48.0% of the women. Breast fluids from nipple aspirations were collected in capillary tubes to be used in cytologic and biochemical studies. Fluid aspirates were obtained most often from Caucasians (70.2% of 225 women) and least often from Chinese (24.1% of 236 women). The percentage of secretors was found to decrease after age fifty in Caucasian and Mexican women, with a greater decrease in Chinese women. Menstrual activity and menopause were found to decrease the number of secretors in Caucasian and Chinese women, while previous pregnancy had no observable effect on frequency of secretions. No significant differences were observed in women who used contraceptives or who received estrogen therapy. In Chinese women, wet cerumen occurred more frequently than did the dry type (34.5% *versus* 15.1%). Women who had undergone prior mastectomies did not show an appreciable decrease in the number of secretors. It is postulated that an increase in secretors of wet cerumen in Orientals is influenced by the alleles determining cerumen secretion.

0504 CANCER OF THE OESOPHAGUS IN BRITTANY: AN INCIDENCE STUDY IN ILLE-ET-VILAINE. (Eng.) Tuyns, A. J. (Int. Agency Res. Cancer, Lyon, France); Massé, G. *Int. J. Epidemiol.* 4(1):55-59; 1975.

A morbidity survey was initiated to confirm the findings of high mortality from esophageal cancer in Brittany and Normandy. A registry, limited to esophageal cancers observed in the *departement* of Ille-et-Vilaine, was established in Rennes. Information was collected from the Regional Cancer Center, departments of pathology and ENT surgery, Social Security files, death certificates, and regional and local hospitals. An accurate tabulation of cases was complicated by repeated reporting. From 1968-1973, 718 cases were entered, 699 males and 49 females. Crude incidence rates were 35:8 and 2:4, resp. and corresponding age-standardized rates were 29:4 and 1:2, resp./100,000 population. The figures for females were within the ranges observed elsewhere in Europe and were not examined further. Forty percent of the tumors occurred in the middle third of the esophagus, 26% in the lower third, 9% stretched over both; 15% were in the upper third, and 8% stretched over the upper and middle thirds. Eighty percent of the tumors examined were squamous cell carcinomas; 7% were adenocarcinomas. There was a high endemicity in the rural areas north of Ille-et-Vilaine with rates reaching 60/100,000. It is suggested that the high incidence of esophageal cancer is related to various etiological factors such as the consumption of local brands of apple cider distillants. Dietary, smoking, and drinking habits in relation to esophageal cancer are now being investigated.

0505 SEROEPIDEMIOLOGIC STUDY OF EPSTEIN-BARR VIRUS INFECTIONS IN A RURAL COMMUNITY. (Eng.) Sumaya, C. V. (Univ. California, Los Angeles Sch. Med.); Henle, W.; Henle, G.; Smith, M. H. D.; LeBlanc, D. *J. Infect. Dis.* 131(4):403-408; 1975.

The prevalence and titers of antibodies to capsid antigen (CA) to Epstein-Barr virus (EBV) were determined in a systematic sample of 109 households in a semirural Louisiana community (462 individuals of Ward 5, St. James Parish) to ascertain its association with various epidemiologic or demographic factors. The interview questionnaire included general vital statistics; medical history; and the testing of exposure to chemicals, other products, and animals. Antibodies to EBVCA were determined by direct immunofluorescence. Titers of antibody to CA $\geq$ 10 were found in 84% of children aged 2 to 5 yr. The prevalence increased with age to 100%. There was a positive but variable correlation of the prevalence of anti-CA reactivity with low socioeconomic status and crowding. An over-representation of high antibody titers to CA was present in individuals with a history of pneumonia and urinary tract infections. Geometric mean antibody titers were the highest in early childhood (7-12 months), lowest in adolescence and young adulthood (11-15 yr), and high in the elderly. Females (all age groups) and tonsillectomized children showed a greater geometric titer than their male and nontonsillectomized counterparts, resp. Antibodies to the early antigen complex were found rarely (8.2%) and only in sera with high antibody titers to CA. In this study, the differences in antibody titers associated with various epidemiologic factors, even if statistically significant, were considered marginal.

0506 EARLY GASTRIC CANCER -- EXPERIENCE IN GERMANY. (Eng.) Elster, K. (Dep. Pathol., Munic. Hosp., Bayreuth, West Germany); Kolaczek, F.; Shimamoto, K.; Freitag, H. *Endoscopy* 7(1):5-10; 1975.

An analysis of 87 cases of early gastric cancers in Germany does not demonstrate significant differences from Japanese findings. Macroscopic types, histological patterns and topographic distributions were similar. A transparent plastic film was placed on the extended, fixed stomach and the lesion contours were drawn. Outlines were transferred to graded paper and excised blocks were marked. Thus, the histological section was precisely oriented with respect to the entire lesion. Most cases of early gastric cancer occurred in males between the ages of 40 and 49 yr. Signet ring cell carcinoma was more common at a younger age, while highly differentiated adenocarcinoma predominated in old age. In most cases, diagnosis of the carcinoma was obtained by the first biopsy (69 of 87 cases). The number of diagnoses of early gastric cancer increased along with the number of gastroscopic examinations.

0507 DIET AS AN ETIOLOGICAL FACTOR IN THE DEVELOPMENT OF CANCERS OF THE COLON AND RECTUM. (Eng.) Howell, M. A. (Natl. Cancer Inst., Bethesda, Md.). *J. Chron. Dis.* 28(2):67-80; 1975.

Diet histories of Japanese migrants were compared to cancer mortality and/or incidence rates to determine the role of changing diet in the development of colorectal cancer. Diet histories were obtained through a case-control study and from food consumption data. Case-control studies involved 243 patients with bowel cancer and 486 hospital controls. The effects of the major food groups were analyzed. International food consumption data were obtained from Food Balance Sheets of the Food and Agriculture Organization for 1964-66. National consumption data were obtained from the Department of Agriculture periodic surveys and the American Cancer Society questionnaire begun in 1959. The findings of both sources reinforced each other and supported the case-control study. The evidence suggests that meat, particularly beef, is associated with the development of malignancies of the large bowel. Poultry was probably not associated with the etiology of bowel cancer. Vegetal food sources were independent or tended to be negatively related to intestinal or colonic cancer mortality and incidence.

0508 HODGKIN'S DISEASE, TONSILLECTOMY AND FAMILY SIZE. (Eng.) Gutensohn, N. (Harvard Sch. Public Health, Boston, Mass.); Li, F. P.; Johnson, R. E.; Cole, P. *N. Engl. J. Med.* 292(1):22-25; 1975.

The association of tonsillectomy and Hodgkin's disease was investigated in 77 male and 59 female Caucasian patients between the ages of 15 and 44. The information from Hodgkin's patients on history of tonsillectomy (obtained by questionnaire) was compared with that from two control groups: the 315 living siblings of the patients and the 78 spouses of the married patients. The risk ratio of Hodgkin's disease among tonsillectomized persons was 3.1 on the basis of case-spouse comparison and 1.4 on the basis of case-sibling comparison. Case-sibling analysis was repeated according to sibship size. Increased disease risk was associated with tonsillectomy only within 37 sibships of size two. Variation of risk ratio with sibship size was expressed. The range of association implied that the relation of tonsillectomy and Hodgkin's disease was either noncausal or complex and modified by family size. Risk of Hodgkin's disease increased as sibship size decreased. This suggests that a cause of Hodgkin's disease was correlated with childhood social class.

0509 LUNG CANCER AMONG BLACK AND WHITE MIGRANTS IN THE U.S.: ETIOLOGICAL CONSIDERATIONS. (Eng.) Mancuso, T. F. (Grad. Sch. Public Health, Univ. Pittsburg, Pa.); Sterling, T. D. *J. Natl. Med. Assoc.* 67(2):106-111,102; 1975.

An investigation concerning the critical examination of the interaction between environmental and host factors was discussed, supported by data obtained from various migrant populations. Having previously noted the consistent rise in lung cancer mortality among blacks, particularly in males, a detailed examination of Ohio residents was pursued. The age-specific death rates and age-adjusted mortality rates per 100,000 were tabulated. No difference was found between the white and black males born in Ohio; however, white migrants experienced a 50% increase, and black migrants a 100% increase in age-adjusted mortality. Similar but less striking differences were found in females, while the findings indicated that the dramatic increase in lung cancer in black males is limited to migrants. In considering specific hypotheses, it was deduced that smoking is completely unrelated to the difference in lung cancer rates. Factors of general malnutrition and related disease were virtually unexplored. No major role could be assigned to exposure to agricultural chemicals during childhood, although altered resistance to subsequent environmental carcinogenic agents could not be discounted. Environmental chemicals, plus associated factors of urbanization and air pollution appear the most decisive factors; the higher rates in males support the occupational industrial factor concept. The hypothesis concerning environmental stresses after migration was supported *via* analysis of data from nonwhite coke plant workers. Thirty-three of 35 deaths among 2,543 workers through 1966 occurred in men born in the South. Thus, the findings support the hypothesis that lung cancer, at least among blacks, may be largely a result of migration and occupational exposure to chemical dusts and fumes in the industrial environment. In addition, region of birth in the U.S., migration, and subsequent industrial employment may be important variables.

0510 MEASURING THE BENEFIT OF REDUCED EXPOSURE TO ENVIRONMENTAL CARCINOGENS. (Eng.) Gail, M. (Natl. Inst. Health, Bethesda, Md.). *J. Chronic Dis.* 28(3):135-147; 1975.

Three figures of merit to measure the benefits of reducing the exposure dose of environmental carcinogens are proposed, and a method for the estimation of these figures from life table data under competing risk assumptions is given. From the table, graphic estimates of survival were drawn. Median survival age was 77.4, 72.3, and 67.9 yr, resp., for persons smoking 0, 10-20, and over 39 cigarettes/day. The median age of survival for nonsmokers was 9.5 yr longer than for those smoking over 39 cigarettes/day and 5.1 yr longer than for people smoking 10-20 cigarettes/day. The risk of developing cancer by age 65 increased sharply with increasing cigarette dose. The analysis suggests that reduced smoking would increase life expectancy and decrease the probability of contracting lung cancer, but would have little impact on future cancer victims.

0511 ANGIOSARCOMA OF THE LIVER IN VINYL CHLORIDE/POLYVINYL CHLORIDE WORKERS. (Eng.) Lloyd, J. W. (Natl. Inst. Occup. Saf. Health, Rockville, Md.). *J. Occup. Med.* 17(5):333-334; 1975.

Review of recent data on cases of liver angiosarcoma illustrated a clustering within recent years. Although the high risk of the disease for polymerization workers had previously gone unnoticed, an exact estimate of the incidence of liver angiosar-

coma among the workers could not yet be determined, pending exposed-to-risk figures. Approximately 5,600 men are currently employed in 36 U.S. facilities, with 15 cases of liver angiosarcoma reported to date. Greater than 70% of the cases were initially employed in polymerization work more than 20 yr ago, setting an average latent period of 19 yr. The age at diagnosis of angiosarcoma among both vinyl chloride workers and polymerization workers was considerably lower than that of the general population, suggesting the possibility of a common etiology. Preliminary findings from continuing epidemiologic investigations indicated that polymerization workers may also be at high risk for other malignant neoplasms, particularly of the brain. It remains to be determined whether exposure to lower levels of vinyl chloride also represents excessive risk for liver angiosarcoma or other malignancies.

0512 TRANSMISSION OF HUMAN LEUKAEMIA TO NONHUMAN PRIMATES. (Eng.) Lapin, B. A. (USSR Acad. Med. Sci., Sukhumi); Yakovleva, L. A.; Indzhiia, L. V.; Agrba, V. Z.; Tsiripova, G. S.; Voevodin, A. F.; Ivanov, M. T.; Djatchenko, A. G. *Proc. R. Soc. Med.* 68(3):141-145; 1975.

The development of malignant lymphoma in stumptail monkeys (45 monkeys, 2-4 yr-old, *Macaca arctoides*) and baboons (21 baboons, *Papio hamadryas*) following the injection of human leukemic whole blood and plasma filtrate was studied. Leukemic blood was obtained from 32 patients. Injections were made parenterally, mostly i.p., 20-30 ml of blood or plasma for primary injection and 3-6 ml for monkey-to-monkey passages. Controls used were: heat-inactivated human leukemic blood (in six monkeys), healthy human blood (eight monkeys), heat-inactivated infected monkey blood (12 monkeys), heat-inactivated monkey spleen cells (four monkeys), noninfected monkey blood (four monkeys), and noninfected monkey spleen cells (four monkeys). Control and experimental animals underwent clinical and hematological studies, bone marrow aspirates, and bone marrow and lymph node biopsies. Inoculation with human leukemic blood or the filtrate caused a viral disease with the characteristics of malignant lymphoma of mixed type. The main symptom was splenomegaly in the monkey and skin lesions and splenomegaly in the baboon. The disease was passed in subsequent passages. The virus was isolated and identified as an oncornavirus of C-type (in 32 of 45 monkeys) by the morphological appearance, buoyant density (1.16 g/cm^3 in sucrose and 1.21 g/cm^3 in *cesium chloride*), and by the presence of 60-70S RNA and RNA-dependent DNA polymerases. This virus is believed to differ from other known oncornaviruses of mammals. It is also postulated that this virus can be transmitted both horizontally and vertically.

0513 A METHOD TO STUDY CELL PROLIFERATION KINETICS IN HUMAN GASTRIC MUCOSA. (Eng.) Hansen, O. H. (Bispebjerg Hosp., Copenhagen, Denmark); Pedersen, T.; Larsen, J. K. *Gut* 16(1):23-27; 1975.

An autoradiographic method for the study of cell proliferation kinetics in small endoscopic biopsies of human gastric mucosa is presented. Biopsies were taken from the antral (1 biopsy, 2 cm above the pylorus on the lesser curvature) and fundic (2 biopsies, high on the greater curvature) stomach in each of 36 randomly selected patients (20 women and 16 men) through a fiber-gastroscope and incubated in culture medium containing a DNA precursor (^{3}H-thymidine). Autoradiographs were prepared by the dipping technique. The number of labeled cells and the total number of cells in all cross sections of foveolae containing one or more labeled cells were counted. The percentage of labeled cells in the progenitor region (labeling index, LI) was estimated. To reduce the possibility of overestimation when only cross sections with labeled cells were considered, a correction formula was applied. Observer error was minimal and the results were highly reproducible. No correlation was found between the LIs in the antral and fundic mucosa, indicating different growth patterns in the two types of gastric mucosa. Increased epithelial proliferation was found in fundic mucosa from patients with gastric cancer and atrophic gastritis. This procedure allowed the comparison of the fraction of cells in phases of DNA synthesis in human antral and fundic mucosa.

0514 POLYAMINES: A HIGH CORRELATION WITH CELL REPLICATION. (Eng.) Heby, O. (Univ. Calif. Med. Cent., San Francisco, Calif.); Marton, L. J.; Wilson, C. B.; Martinez, H. M. *FEBS Lett.* 50(1): 1-4; 1975.

The changes in cellular polyamine content accompanying alterations in cell growth patterns were studied to determine if they are related to growth rate. An *N*-nitrosomethylurea-induced rat brain tumor was cultivated *in vitro*. Tumor cells were trypsinized during intervals of the seven day growth period. Supernatant aliquots were used for polyamine analysis. Maximal spermidine content coincided with exponential growth and decreased when cell division ceased. The spermidine/spermine ratio showed a direct linear correlation with specific growth rate, indicating that spermidine accumulation is associated with cell replication. The authors also suggest the possible use of polyamine determinations in the qualitative and quantitative evaluation of the efficacy of various modes of chemotherapy.

0515 THE EPIDEMIOLOGY OF CANCER IN OKLAHOMA: EXAMINATION OF CRAMER'S HYPOTHESIS. (Eng.) Asal, N. R. (Univ. Oklahoma Health Sci. Cent., Oklahoma City); Anderson, P. S., Jr. *South. Med. J.* 68(2):193-201; 1975.

0516 CANCER STATISTICS, 1975. (Eng.) Anonymous. *CA.* 25(1):8-21; 1975.

0517 THE INCIDENCE AND MORTALITY RATES FOR LARYNGEAL CANCER FROM TOTAL CANCER REGISTRIES. (Eng.) Barclay, T. H. C. (Saskatchewan Cancer Commission, Canada); Rao, N. N. *Laryngoscope* 85(2):254-258; 1975.

0518 LEUKAEMIA AND LYMPHOMA LINKED BY PRIOR SOCIAL CONTACT. (Eng.) Pinkel, D. (Milwaukee Child. Hosp., Wis.); Bahn, A. K. *Lancet* 1(7903):393; 1975.

0519 SOME REFLECTIONS ON CARCINOMA OF THE PROSTATE AT THE WEST VIRGINIA UNIVERSITY MEDICAL CENTER. (Eng.) Kandzari, S. J. (West Virginia Univ. Med. Cent., Morgantown); Milam, D. F. *W. Va. Med. J.* 71(3):59; 1975.

0520 RECTAL CARCINOMA. A TWO PART STUDY OF TUMOUR PROLIFERATION AND HOST CELLULAR IMMUNITY. (Eng.) Bone, G. (R. Victoria Infirm., Newcastle Upon Tyne, England); Camplejohn, R. S. *Am. J. Proctol.* 26(1):65-76; 1975.

0521 SCREENING FOR MEDULLARY CARCINOMA OF THE THYROID. (Eng.) Telenius-Berg, M. (Univ. Hosp., Lund, Sweden); Almqvist, S.; Hedner, P.; Ingemansson, S.; Tibblin, S.; Wasthed, B. *Lancet* 1(7903):390-391; 1975.

0522 INCREASED INCIDENCE OF ADENOCARCINOMA OF UTERINE CERVIX. (Eng.) Davis, J. R. (Arizona Med. Cent., Univ. Arizona, Tucson); Moon, L. B. *Obstet. Gynecol.* 45(1):79-83; 1975.

0523 EPIDEMIOLOGIC STUDY ON THE ROLE OF TOBACCO IN THE CAUSATION OF HUMAN CANCERS. (Fre.) Flamant, R. (Institut Gustave-Roussy, 16 bis, avenue Paul-Vaillant-Couturier, F 94800 Villejuif, France); Pen, Y. *Bull. Cancer (Paris)* 62(1):93-102; 1975.

0524 EARLY DETECTION OF CANCER OF THE CERVIX UTERI. RESULTS FROM THE BADEN-WURTTEMBERG CANCER REGISTRY 1969-1973. (Ger.) Neumann, G. (7 Stuttgart 1, Schickhardtstrasse 35, West Germany). *Fortschr. Med.* 93(4):139-140, 168; 1975.

0525 EPIDEMIOLOGICAL AND CLINICAL SURVEY OF MAMMARY CANCER PATIENTS BY QUESTIONNAIRE METHOD. (Hun.) Pentek, Z. (Rontgen Osztaly, Tolna Megyei Tanacs "Balassa Janos" Korhaz-Rendelointezet, Kaposvar, Hungary); Tabar, L.; Szentgali, G. *Mag. Onkol.* 19(2):82-88; 1975.

0526 GENEVA TUMOR REGISTER: CANCER INCIDENCE IN GENEVA [abstract]. (Fre.) Riotton, G. (Geneva, Switzerland); Raymond, L. *Schweiz. Med. Wochenschr.* 105(17):539; 1975.

0527 QUANTITATIVE STUDIES OF THE GROWTH AND REJECTION OF ALLOGENEIC TUMOUR CELLS IN MOUSE CEREBROSPINAL FLUID: ELIMINATION IN THE ABSENCE OF H-2 DIFFERENCES. (Eng.) Doherty, P. C. (John Curtin Sch. Med. Res., Canberra, Australia); Zinkernagel, R. M. *Clin. Exp. Immunol.* 19(2):355-366; 1975.

See also:

* (Rev): 0015, 0016, 0017, 0018, 0019, 0026, 0030, 0033, 0054, 0055, 0056, 0057
* (Chem): 0084, 0118, 0150, 0152, 0172
* (Phys): 0195
* (Viral): 0237
* (Path): 0397

0528 ONCOGENESIS BY INTERSPECIFIC INTERACTION OF MALIGNANT MURINE AND NON-MALIGNANT HAMSTER CELLS *IN VITRO*. (Eng.) Goldenberg, D. M.; (Univ. Kentucky Med. Cent., Lexington); Pavia, R. A. *Int. J. Cancer* 15(2):282-300; 1975.

A clone of Cloudman S91 murine melanoma was fused *in vitro* with non-malignant hamster cheek pouch cells (HCP) by means of lysolecithin; and the putative hybrid progeny cells; HCP-MM, were found to be highly malignant in hamsters, but not in appropriate mice. A malignant clone of HCP-MM cells was shown to have hamster species-specific surface antigens (as demonstrated by immunofluorescence and the cytotoxic antibody) and hamster-like lactate dehydrogenase and NAD-dependent malate dehydrogenase isoenzyme profiles. Nevertheless, chromosomes similar to those of both murine and hamster parental cells could be distinguished in cells of the malignant clone and in hamster tumor grafts by trypsin-Giemsa banding. A majority of the murine chromosomes, however, appeared to be lost. This study indicates that a murine melanoma previously found untransplantable in hamsters could produce a highly malignant and lethal tumor for hamsters after being mixed *in vitro* with non-malignant hamster cells in the presence of a fusing chemical. It is not certain whether the production of transformed cells *in vitro* and of highly malignant tumors in the hamster required heterosynkarion formation between the murine melanoma and hamster cheek pouch cells. It is suggested that the presence of the murine melanoma, and possibly the interaction of its genome with non-malignant hamster cells, was implicated in this process.

0529 CELL DENSITY-DEPENDENT GROWTH IN AGAR OF BONE MARROW CELLS FROM TUMOR-BEARING BALB/c MICE IN THE ABSENCE OF A COLONY-STIMULATING FACTOR. (Eng.) Nooter, K. (Radiobiol. Inst. Rijswijk, Netherlands); Bentvelzen, P. *Cancer Res.* 35(1):117-121; 1975.

The ability of bone marrow from BALB/c mice with various types of tumors to grow in agar in the absence of colony-stimulating factor (CSF) was studied. Bone marrow cells from female mice with Rauscher murine leukemia virus (RLV)-induced erythroblastosis, myeloid leukemia and lymphosarcoma, and with murine mammary tumor virus (MTV)-induced mammary tumors were added to agar in the presence and absence of CSF. Bone marrow cells from mice with myeloid leukemia, lymphosarcoma, erythroblastosis, or mammary tumor produced small clusters in the semisolid agar cultures in the absence of specific CSF. This spontaneous growth was observed only when high cell numbers (5×10^5 cells/ml) were plated, and the phenomenon was encountered only when the mice had an elevated number of mature or immature granulocytes in the peripheral blood. Removal of the adherent cells from the bone marrow did not abolish the spontaneous growth, indicating that this CSF independency was not due to a high number of colony-stimulating cells in the bone marrow of the cancerous mice. Feeder layers of bone marrow cells from the diseased animals had no higher colony-stimulating activity than did normal bone marrow cells, which excluded the possibility that the spontaneous growth was due to a high endogenous stimulating activity of the bone marrow from the tumor-bearing mice. The active effect of closely packed cells at high cell densities may be the factor which generates some growth in the case of diseased animals.

0530 LACK OF RELATIONSHIP BETWEEN ACTIVITY OF CHROMATIN-BOUND PROTEINASE AND CELL GROWTH RATES. (Eng.) Chae, C. B. (Dep. Biochem., Univ. North Carolina, Chapel Hill); Smith, M. C.; Morris, H. P. *Biochem. J.* 146(1):281-283; 1975.

The activity of chromatin-bound proteinases of Morris hepatomas with different growth rates was determined. Chromatins isolated from normal or host liver and hepatomas were incubated in 0.3 M NaCl and 10 mM Tris-HCl at 37 C for different periods of time, and the amount of lysine-rich (F1) histone remaining was determined from the densitometric tracings of histone gels. The chromatin-bound proteinases from normal liver and a fast-growing hepatoma (7777) degraded F1 histone at the same rate (40% in two hr). In contrast, the F1 histone from chromatin of two slower-growing hepatomas (7794A and 9121) was degraded at a considerably higher rate (65% in two hr) than the F1 histone from host liver chromatin. However, the F1 histone in another, slower-growing hepatoma (7800) was completely degraded in 90 min. In the presence of salt, chromatin isolated from Ehrlich ascites carcinoma, a fast-growing tumor, had no apparent proteinase activity. Thus, there appears to be no strict relationship between the activity of proteinase and the growth rates of Morris hepatomas.

0531 EFFECT OF A NORMAL SERUM PROTEIN ABSENT FROM HEPATOMA-BEARING ANIMALS ON CELL CULTURES. (Eng.) Dolan, M. L. (Sch. Med., Univ. Pittsburgh, Pa.); Coetzee, M. L.; Spangler, M.; Ove, P. *J. Natl. Cancer Inst.* 54(1):163-169; 1975.

A serum factor separated from normal rat serum using DEAE-cellulose chromatography was studied for its effects on growth, ^{3}H-thymidine and ^{3}H-leucine incorporation in 3T3 and L cells in culture. The occurrence of the factor in the serum of BUF rats bearing hepatoma 7777 was also investigated. Serum from hepatoma-bearing rats did not contain the factor when the hepatomas were greater than or equal to liver weight. The amount of factor in the serum of rats bearing hepatomas was found to be inversely proportional to the size of the tumor. ^{3}H-thymidine and ^{3}H-leucine incorporation and growth of 3T3 and L cells were supported by 10% calf serum or 5% rat serum in the medium. Growth and label incorporation were decreased by 50% if serum from rats with hepatomas was used. Growth and label incorporation could be returned to 70-90% of that of normal rat serum, if purified factor was added to serum from rats bearing hepatomas. Following release from contact inhibition, ^{3}H-thymidine uptake was less in 3T3 cells maintained in 5% serum from hepatoma-bearing rats, but ^{3}H-leucine incorporation was the same as that in 3T3 cells grown in 5% normal serum. Addition of

factor increased ^{3}H-thymidine incorporation in cells maintained in hepatoma serum. It is suggested that there may be a direct link between this factor and growth and metabolism of normal and neoplastic cells.

0532 BONE-MARROW-PROLIFERATION PATTERNS IN ACUTE MYELOBLASTIC LEUKAEMIA DETERMINED BY PULSE CYTOPHOTOMETRY. (Eng.) Hillen, H. (Dep. Intern. Med., Univ. Nijmegen, Netherlands); Wessels, J.; Haanen, C. *Lancet* 1(7907):609-611; 1975.

A study was undertaken to compare pulse cytophotometry (PCP) with autoradiography in evaluating cell proliferation patterns in bone marrow cells in acute myeloblastic leukemia (AML). In 19 normal bone-marrow samples 63% of the cells were in the G_1 phase, 26% in the S phase and 7.5% in the G_2+M phase. The percentage of S phase cells correlated well between the two methods, with the nonparametric rank correlation coefficient being highly positive ($r = 0.95$, $P < 0.001$). Patients with untreated AML had a lower percentage of cells in S and G_2+M phases when compared to controls. The patients with the lowest amount of proliferating S and G_2+M phase cells did not benefit from treatment unless the level was more than 15%. PCP provides rapid information about bone marrow proliferation before, during and after chemotherapy and may improve treatment schedules and prognosis of acute leukemia.

0533 ADENOSINE-3',5'-CYCLIC MONOPHOSPHATE LEVELS AND ADENOSINE-3',5'-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE ACTIVITY IN METASTASIZING AND NON-METASTASIZING RAT MAMMARY CARCINOMAS. (Eng.) Chatterjee, S. K. (Roswell Park Mem. Inst., Buffalo, N.Y.); Kim, U. *J. Natl. Cancer Inst.* 54(1):181-186; 1975.

The adenosine-3',5'-cyclic monophosphate phosphodiesterase (cPDE) activity in the homogenates of six spontaneously metastasizing, nonimmunogenic, glycocalyx-shedding rat mammary carcinomas (MT) was assayed and compared with four histologically and growth rate-matched nonmetastasizing, immunogenic MT. The levels of this enzyme were 2.5 times higher in the nonmetastasizing tumors. To rule out the possibility of the presence of inhibitors or stimulators of cPDE, homogenates from a nonmetastasizing and from a widely metastasizing tumor were mixed. cPDE from both nonmetastasizing and metastasizing MT showed two apparent K_m and two corresponding V_{max}. The activity of the enzyme at concentrations of 1 μM (low K_m) and 100 μM (high K_m) adenosine-3',5'-cyclic monophosphate (cAMP) decreased in parallel with increasing metastasizing capacity. About 50% of the low and the high K_m cPDE was in the cytosol in both groups, whereas the rest was particulate. The proportion of low (3 μM) and high (50 μM) K_m activity was similar in all the fractions except in the plasma membranes of the metastasizing tumors, where the percent of low K_m enzyme was three times higher than that of the high K_m enzyme (1.8 *versus* 0.6). The steady-state levels of cAMP were 1.3-2.0 times higher in the metastasizing tumors (inversely proportional to their cPDE activities). It is suggested that when chemically-induced MT acquire metastasizing capacity or lose immunogenicity by shedding the glycocalyx, the endogenous cAMP levels increase due to the decreased cPDE activity in these cells.

0534 CHOLERA TOXIN ACTIVATION OF ADENYLATE CYCLASE IN CANCER CELL MEMBRANE FRAGMENTS. (Eng.) Bitensky, M. W. (Yale Univ. Sch. of Medicine, 310 Cedar St., New Haven, Conn. 06510); Wheeler, M. A.; Mehta, H.; Miki, N. *Proc. Natl. Acad. Sci. USA* 72(7):2572-2576; 1975.

Activation of adenylate cyclase [ATP pyro-phosphate-lyase (cyclizing)] by cholera toxin (84,000 daltons) was demonstrated in suspensions of plasma membrane fragments prepared for sarcoma 180 and other mouse ascites cancer cells. The activation of adenylate cyclase was mediated by a macromolecular cyclase activating factor (MCAF), which had a sedimentation constant of 2.7 S and a molecular weight of about 26,000. MCAF is derived from, and may be identical to the "A fragment" of cholera toxin. Generation of MCAF depends on prior interaction of cholera toxin with either dithiothreitol, NADH, NAD, or a low-molecular-weight component. Subsequent exposure of this pretreated cholera toxin to cell membranes from a variety of mouse ascites cancer cells was followed rapidly by the appearance of MCAF, which no longer required dithiothreitol, NADH, or NAD for the activation of ATP. Activation of adenylate cyclase by MCAF in ascites cancer cell membrane fragments was not reversed by repeated washing of these membrane fragments. ATP in normal cell membrane fragments failed to respond either to cholera toxin or MCAF in the presence of dithiothreitol. In constrast, the adenylate cyclase in membrane fragments from five ascites cancer cells responded to either MCAF or native cholera toxin preincubated with dithiothreitol, NADH, or NAD.

0535 GROWTH AND DEATH OF DIPLOID AND TRANSFORMED HUMAN FIBROBLASTS. *(Eng.)* Holliday, R. (Natl. Inst. Med. Res., London, England). *Fed.Proc.* 34:51-55; 1975.

Three possible explanations are presented for the differences in growth potential between human diploid fibroblasts of finite life-span and permanent transformed lines: 1) Only diploid cells have a molecular clock mechanism which counts cell division prior to senescence. Two hypothetical examples of such mechanisms are described; however, the available evidence argues against a clock mechanism for aging in fibroblasts. 2) Cells become committed with a given probability to a slow buildup in protein errors, which leads after many divisions to a lethal error catastrophe. Speeding up the rate at which the error catastrophe develops, as may occur in transformed cells, can convert a population of finite life-span to one with infinite growth. 3) The growth rate of diploid cells may not depend on the limiting concentration of any one protein. If so, cells with a low level of errors will not have a reduced generation time, and there will be no selection against them. On the other hand, the uncontrolled growth of transformed cells may be reduced

in rate by the presence of faulty proteins, so that there is continuous selection for those with the fewest errors. The analogous problem of the mortality of somatic cells and the immortality of the germ line is also briefly discussed.

0536 THE PINEAL AND NEOPLASIA. (Eng.) Buswell, R. S. (Univ. Colorado Med. Cent., Denver). *Lancet* 1(7897):34-35; 1975.

The possibility that the pineal gland contains a substance which inhibits tumor growth was investigated. Two groups of BALB/c mice were pretreated with 100 μg of melatonin in a propylene glycol-saline solution or with propylene glycol-saline alone. One-half hr later, 10^3 LSTRA cells (a transplantable leukemia) were inoculated i.m.-s.c. The respective melatonin or control solutions were then inoculated on a daily basis until the animals were sacrificed 14 days later. The tumors were weighed and the two groups were compared. In the melatonin-treated group, five mice developed tumors as compared with 18 control mice; the mean weights of the tumors were 44.0 mg and 242.8 mg, resp. This inhibitory effect of melatonin on tumor development is considered significant. The relation between the pineal gland and melatonin in human malignancy remains unexplored. It is suggested that, with the advent of an assay for urinary melatonin, the significance can be determined.

0537 DIFFERENTIATION IN ERYTHROLEUKEMIC CELLS AND THEIR SOMATIC HYBRIDS. (Eng.) Orkin, S. H. (Natl. Inst. Child Health Hum. Dev., Bethesda, Md.); Harosi, F. I.; Leder, P. *Proc. Natl. Acad. Sci. USA* 72(1):98-102; 1975.

Uniformity in the extent of differentiation in cells within cloned lines was studied in erythroleukemic cells and their somatic hybrids. Microspectrophotometry, cDNA probes, and DNA x RNA and DNA x DNA hybridization assays were used to study globin gene expression and differentiation in stock erythroleukemic cell lines (T3-C12 and GM 86), in somatic hybrid cells formed between similar erythroleukemic lines, in somatic hybrids between phenotypically different erythroleukemic lines, and in hybrids between erythroleukemic cells and A9 mouse fibroblasts. Relative differences in globin gene expression within and between nonhybridized erythroleukemic clones tended to reflect differences in the proportion of cells participating in differentiation rather than to indicate uniform differences in the extent to which all cells in these clones underwent differentiation. It is proposed that this phenotypic characteristic of a clone be called its probability of differentiation, a property which reflects the likelihood that a cell within a given clone will undergo differentiation under given conditions. This characteristic was preserved in subclones of the cloned lines, indicating that the probability of differentiation in these may be controlled by relatively stable genetic or epigenetic factors. Somatic hybrids between similar erythroleukemic lines resembled the parental lines with respect to their pattern of erythroid differentiation, and in hybrids between dissimilar erythroleukemic lines, the extent of differentiation in the untreated and induced states was uncoupled; somatic hybrids between mouse fibroblasts and erythroleukemic cells demonstrated apparent extinction of globin expression. The results suggest that spontaneous and induced probabilities of differentiation may be determined at different steps leading to a common pathway of globin gene expression.

0538 MALIGNANT HEMANGIOENDOTHELIOMAS PRODUCED BY SUBCUTANEOUS INOCULATION OF BALB/3T3 CELLS ATTACHED TO GLASS BEADS. (Eng.) Boone, C. W. (Natl. Cancer Inst., Bethesda, Md.). *Science* 188(4183):68-70; 1975.

The phenomenon of anchorage-dependence (the inability to divide *in vitro* unless attached to a solid substrate) was studied *in vivo*, as well as the growth and production of tumors by Balb/3T3 cells attached to a solid substrate. Balb/c mice were s.c. inoculated with an average of 15,400 Balb/3T3 cells attached to two glass beads 3 mm in diameter. Control mice were inoculated with glass beads which had been incubated in tissue culture mediium without Balb/3T3 cells, or with Balb/3T3 cells alone. After eight weeks, all mice inoculated with Balb/3T3 cells attached to glass beads had developed large, blood-filled tumors, which proved to be hemangioendotheliomas. The inoculation of Balb/3T3 cells alone or beads alone produced no such tumors. *In vitro*, the tumor cells had a high plating efficiency and resembled Balb/3T3 cells in their morphology. When inoculated s.c. into other mice, the tumor cells (in doses of 10^4 cells) produced tumors which appeared grossly and microscopically similar to the original tumors. A complete repeat experiment resulted in the production of hemangioendotheliomas in 17/25 mice inoculated s.c. with Balb/3T3 cells attached to glass beads. The results suggest that the Balb/3T3 cell is a vascular endothelial cell which should not be used as the standard for nontumorigenic mouse cells. The reassessment of the current concept that post-confluence inhibition of cell division, low saturation density, and anchorage-dependence (all properties of the Balb/3T3 cell) are characteristic *in vitro* properties only of nonneoplastic cells is advised.

0539 EARLY AND LATE CHANGES IN NONHISTONE CHROMATIN PROTEINS ACCOMPANYING RAT LIVER REGENERATION. (Eng.) Yeoman, L. C. (Baylor Coll. Med., Houston, Tex.); Taylor, C. W.; Jordan, J. J.; Busch, H. *Cancer Res.* 35(5):1249-1255; 1975.

Changes in nonhistone chromatin proteins in early and later stages of growth were studied using two-dimensional polyacrylamide gel electrophoresis. Regenerating rat liver nonhistone chromatin proteins were examined at 1, 5, 18, 24, and 48 hr after partial hepatectomy. After one hr there was a marked decrease in density of protein spots C18 and CQ while density decreased less in spots Bp and B24. Spots

CBL and C13 increased in density after one hr. After five hr the density of spots C18, CQ and CBL remained unchanged; spots B24 and Bp regained density while spots C13 and CN decreased slightly. At 18 hr there was an increased density in spots C18, CQ, C25 and CT with a decrease in spots BA and CBL. The patterns after 24 hr showed spots C18 and CQ smaller; Bp remained dense and spots BA, CBL, C13 and CN decreased in density. The same conditions prevailed after 48 hr except that spot B24 increased in density. In general, the chromatin fraction II protein/DNA mass ratio did not change significantly during the experimental period. However, the decrease in protein spot BA may be important in the growth of normal and neoplastic cells.

0540 EVIDENCE FOR HETEROGENEITY OF RIBOSOMES WITHIN THE HELA CELL. (Eng.) McConkey, E. H. (Dep. Mol. Cell. Dev. Biol., Univ. Colorado, Boulder); Hauber, E. J. *J. Biol. Chem.* 250(4):1311-1318; 1975.

Ribosomal proteins obtained from 40S and 60S subunits of native subunits, single ribosomes, free polysomes and membrane-bound polysomes were separated using high resolution polyacrylamide gel electrophoresis in order to examine the question of HeLa cell-ribosomal heterogeneity. All of the 40S subunits contained 17 proteins in common. The 40S subunits from single ribosomes were characterized by an absence of protein of molecular weight greater than 35,000 daltons. Native 40S subunits contained substantial amounts of protein of molecular weight greater than 35,000 daltons, which varied with each preparation. Free polysome 40S subunits contained a large number of high molecular weight proteins, none of which corresponded with native 40S protein. The 40S subunit proteins from bound polysomes were nearly all less than 35,000 daltons. Many of these differences may be due to separation techniques used prior to electrophoresis. All of the 60S subunits contained 35 regularly occurring components; however, 60S membrane-bound polysome subunits lacked a protein of about 41,000 daltons and contained another protein of about 38,000 daltons not present in the other preparations. It is suggested that these two proteins may play a role in the binding of 60S subunits to the endoplasmic reticulum.

0541 COMPARISON OF RIBOSOMAL PROTEINS FROM NEOPLASTIC AND NON-NEOPLASTIC CELLS: RESOLUTION BY TWO-DIMENSIONAL GEL ELECTROPHORESIS. (Eng.) Subramanian, A. R. (Massachusetts Gen. Hosp., Boston); Gilbert, J. M.; Kumar, A. *Biochim Biophys Acta* 383(1):93-96; 1975.

Ribosomal proteins obtained from functionally active membrane-free ribosomes of rat forebrain, mouse neuroblastoma C-1300 and HeLa cells were resolved using a two dimensional gel electrophoretic procedure capable of good resolution of high molecular weight acidic proteins. Comparison of the patterns obtained revealed four major differences. One spot was strong in the forebrain and weak in the tumor cells; another spot was weak in the forebrain and strong in the neoplastic cells. Two other spots were observed to be displaced in both neuroblastoma and HeLa cell protein patterns, apparently due to an increase in net positive charge or a decrease in molecular weight. Four additional spots were seen in the neuroblastoma ribosomal protein pattern which were not seen in the forebrain or HeLa cell patterns. It is concluded that, while ribosomal protein patterns from free ribosomes of neoplastic and non-neoplastic cells are quite similar, enough differences are observed to clearly identify the source of the ribosomes.

0542 DNA-BINDING PROTEINS IN YOUNG AND SENESCENT NORMAL HUMAN FIBROBLASTS. (Eng.) Stein, G. H. (Stanford Univ. Sch. Med., Calif.). *Exp. Cell Res.* 90(2):237-248; 1975.

The relationship of DNA binding proteins (DBP) to the replicative state in young and senescent WI38 human fibroblast cells was examined to determine if DBP play a role in the regulation of cell replication. The amounts of DBP in different cell populations were measured by adding radioactive amino acid precursors to the cells 40 hours before isolation of the DBP using DNA-cellulose chromatography and polyacrylamide gel electrophoresis. Five proteins, P5b (87,000 daltons), P6a (50,000), P8 (33,000), P9 (28,000) and P10 (25,000) were labeled more in replicating than in stationary cells. Two proteins P5c (72,000 daltons) and P12 (18,000) were labeled more in the stationary phase cells. Several DBP of high molecular weight were labeled to a greater extent in stationary phase cells, and these were partially characterized as protocollagen and collagen. Senescent WI38 cells, near or at the end of their *in vitro* lifespan, showed increased proportions of P8 in the stationary phase when compared to WI38 cells at early doubling levels. In the growth phase, P8 amounts to more than 1% of the total soluble protein in young cells, and binds preferentially to single stranded DNA. It is concluded that WI38-P8 is comparable to a previously-characterized P8 from human SB and mouse 3T6 cells. It is suggested that increased amounts of P8 in senescent cells may be involved in the irreversible inhibition of replication at the end of the *in vitro* lifespan.

0543 NATURE OF THE COLLAGEN SYNTHESIZED BY A TRANSPLANTED CHONDROSARCOMA. (Eng.) Smith, B. D. (Natl. Inst. Dent. Res., Bethesda, Md.); Martin, G. R.; Miller, E. J.; Dorfman, A.; Swarm, R. *Arch. Biochem. Biophys.* 166(1):181-186; 1975.

The collagen extracted from a chondrosarcoma maintained by serial transplantation in BUF/N or BUF/NMai rats was characterized by carboxymethyl-cellulose chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and molecular sieve chromatography. The initial chondrosarcoma was derived from a spontaneous bone tumor in a Sprague-Dawley rat. The collagen was almost exclusively type II collagen, the cartilage-specific protein. Since this collagen is a major component of the tumor (40% of total protein), but not a component of the tissue at the transplantation site, the tumor cells

must secrete cartilage collagen and must retain the differentiated characteristic of cartilage cells. It is possible that the chondrosarcoma arose from the transformation of an immature cartilage cell.

0544 MODIFICATION OF NUCLEAR RESTRICTION *IN VITRO* BY PLASMA FROM TUMOR-BEARING ANIMALS. (Eng.) Schumm, D. E. (Ohio State Univ. Coll. Med., Columbus); Webb, T. E. *J. Natl. Cancer Inst.* 54 (1):123-128; 1975.

The plasma of normal and tumor-bearing Sprague-Dawley rats was monitored for regulatory macromolecules. A preliminary assessment was made of the feasability of this assay as a test for diagnosis or tumor detection. The components were monitored by measuring their ability to stimulate messenger RNA (mRNA) release from isolated nuclei in a cell-free system of rat liver nuclei in fortified homologous cytosol. This *in vitro* system showed near-normal *in vivo* nuclear RNA restriction. When added to the assay at a protein concentration of 3.0 mg/ml, dialyzed plasma from rats or mice with chemically induced transplantable or primary tumors stimulated mRNA release from 87% to more than 300% over control plasma from normal rats. Plasma from partially hepatectomized rats stimulated only 26% over control plasma. The test system derived from rat liver seemed to be species nonspecific. It is suggested that regulatory components necessary for normal nuclear restriction *in vitro* may be released from the tumor to the host blood circulation *in vivo*.

0545 CHROMATIN AS A TEMPLATE FOR RNA SYNTHESIS *IN VITRO*. (Eng.) Groner, Y. (Albert Einstein Coll. Med., Bronx, N.Y.); Monroy, G.; Jacquet, M.; Hurwitz, J. *Proc. Natl. Acad. Sci. USA* 72(1):194-199; 1975.

The rate of RNA synthesis from myeloblast chromatin by RNA polymerase B, the structure and size of RNA products formed, and the effect of polyanions on RNA synthesis were studied. Myeloblasts were obtained from avian myeloblastosis virus-infected chickens. The rate of RNA synthesis in the presence of excess chromatin and limiting amounts of RNA polymerase B declined with time until, at 30 min, further ^{3}H-uridine triphosphate incorporation was negligible. Further RNA synthesis was obtained by adding either more enzyme or heparin, a polyanion that has multiple effects on the transcriptional system. The RNA transcribed *in vitro* consisted of short chains that remained in hybrid structure with the template, primarily 5-7S material; the remainder of the product was free RNA of heterogeneous size. The addition of heparin during synthesis caused an increase in the size and amount of free RNA with a concomitant decrease in the proportion of small RNA. The large molecular-wt products formed during chromatin transcription *in vitro* were shown to result from *de novo* synthesis of short RNA chains. The large molecular-wt Rna products were formed by RNA polymerase B in reaction with single-stranded DNA template; with native DNA as template, small molecular-wt products were formed. Native DNA acted as a poor template, while more extensive RNA was formed with single-stranded DNA. Polyanions did not affect the size distribution of the products during transcription of DNA. This fact, plus the observation that the size of RNA products transcribed from chromatin by *E. coli* RNA polymerase was increased by the presence of polyanions, suggest that these compounds may affect chromatin transcription by causing structural modifications of the template.

0546 REGULATION OF AN ASPARTYL-tRNA SPECIES IN BHK CELLS IN CULTURE AND IN SOLID TUMOR FORM. (Eng.) Briscoe, W. T. (M. D. Anderson Hosp., Tumor Inst., Houston, Tex.); Syrewicz, J. J.; Marshall, M. V.; Griffin, A. C. *Biochim. Biophys. Acta* 383(4):441-445; 1975.

Hamster kidney cells (BHK 21/clone 13) grown in culture and in tumor form by s.c. transplantation into Syrian Golden hamsters were compared for content of the various isoaccepting forms of aspartyl-tRNA by reversed phase 5 chromatography. The tRNA from both cultured cells and tumor displayed a four-peak pattern. The major difference in the elution patterns was a substantial increase in peak II and a 20-fold increase in peak IV (late-eluting isoaccepting species) in cultured cells. In tumor cells, the relative amount of peak IV was 1.5-2.8% of the total aspartyl-isoaccepting activity. These results are discussed in relation to the possibility of a derepression or repression of a tRNA modifying enzyme system in tissue culture or by an unknown factor *in vivo*. Further studies using the system described may provide new insight into the role of a specific species of tRNA in regulatory functions that may be involved in differentiation, embryogenesis, or neoplasia.

0547 BIOCHEMICAL PROFILES OF CANCER CELLS: I. COMPUTERIZED ANALYSIS OF MOUSE LEUKEMIC CELLULAR RNA ON POLYACRYLAMIDE GELS. (Eng.) Hacker, B. (Albany Med. Cent. Hosp., N. Y.); Westin, E.; McDermott-Sheedy, B. J.; Doty, C. *Physiol. Chem. Phys.* 7(1):39-52; 1975.

The patterns of total cellular RNA from a subline of mouse leukemia cells resistant to cytosine arabinoside (L1210/Ara C-7) were analyzed on several types of sodium dodecyl sulfate (SDS)-polyacrylamide gels using a computerized spectrophotometric system. The acrylamide gels were specially rinsed in four changes of buffer without SDS and then warmed to 40 C to insure homogeneity and prevent light scattering. Gels were returned to spectrophotometric 'boats' to insure that the buffer-containing end and gel top was scanned first, facilitating the criterion built into the 'prescan' program. Using a calculator-spectrophotometer-integrated system, data was normalized by subtracting individual prescan from scan data. The typical electrophoretic profiles for standard reference markers of 23S, 16S, 5S ribosomal RNA and 4S transfer RNA were found to be inadequate reference markers. The common 16S source was contami-

nated with both high and low molecular weight species which served as added markers. Lower concentrations of bioacrylamide relative to acrylamide (cross-linking) interfered with preparation of optically clean gels. The quantity of any RNA is not necessarily proportional to the height of each peak but rather to the area under the peak; actual calculations were required. It was not possible to use uridylic acid as a reference point for R_f calculations in 20% gels due to conditions necessary for regulating power settings to affect maximum resolution. This 'fingerpoint' method for analyzing RNA metabolism may be a useful biochemical test in leukemia.

0548 SHORT-LIVED MESSENGER RNA IN HELA CELLS AND ITS IMPACT ON THE KINETICS OF ACCUMULATION OF CYTOPLASMIC POLYADENYLATE. (Eng.) Puckett, L. (Dep. Biol. Sci., Columbia Univ., New York, N.Y.); Chambers, S.; Darnell, J. E. *Proc. Natl. Acad. Sci. USA* 72(1):389-393; 1975.

The possible existence in HeLa cells of a short-lived messenger RNA (mRNA) was studied, and the results were considered in terms of their impact on the kinetics of accumulation of cytoplasmic polyadenylate within these cells. Cultured HeLa cells were labeled with (^{3}H)adenine and (^{3}H)guanosine, after which the cells were fractionated and the cytoplasmic and nuclear RNA was extracted. The accumulation of (^{3}H)adenine in the acid-soluble pool and in the nuclear and cytoplasmic poly(A) of these cells indicated that the nuclear poly(A) rose along a curve similar to that of the acid-soluble pool. Pulse-chase experiments with (^{3}H)guanosine in adenine-grown cells revealed that at least 35-50% of the pulse-labeled mRNA had a half-life of about 1-2 hr. Using these data, a mathematical model involving nuclear poly(A) synthesis and conservative transport to the cytoplasm was developed. This model predicts curves similar to those found for the nuclear and cytoplasmic accumulation of poly(A). Thus, there is no necessity on kinetic grounds to invoke either nuclear turnover or cytoplasmic synthesis of poly(A).

0549 MATURATION PATHWAY FOR NOVIKOFF ASCITES HEPATOMA 5.8 S RIBOSOMAL RIBONUCLEIC ACID: EVIDENCE FOR ITS PRESENCE IN 32 S NUCLEAR RIBONUCLEIC ACID. (Eng.) Nazar, R. N. (Baylor Coll. Med., Houston, Texas); Owens, T. W.; Sitz, T. O.; Busch, H. *J. Biol. Chem.* 250(7):2475-2481; 1975.

To confirm the origin of 5.8 S ribosomal RNA (rRNA) subunit in the nucleolar 32 S RNA precursor, specific oligonucleotide sequences of these RNA species were studied. Purified ^{32}P-labeled 5.8 and 28 S rRNA and 32 S RNA were digested with T ribonuclease and the products were fractionated according to chain length by chromatography. Subsequent treatment with alkaline phosphatase showed the 32 S RNA to contain 26 major spots while 28 S and 5.8 S rRNA contained only 16 and 7 spots respectively. Five of the 5.8 S marker oligonucleotides were present in distinct spots in 32 S RNA and absent in 28 S RNA, indicating that 5.8 rRNA is directly derived from the 32 S nucleotide precursor. These results demonstrate a maturation pathway for rRNA species in which 32 S nucleolar RNA is a precursor of 5.8 S rRNA and 28 S rRNA.

0550 REGULATION OF DNA REPLICATION ON SUB-CHROMOSOMAL UNITS OF MAMMALIAN CELLS. (Eng.) Hand, R. (Dept. Med., McGill Univ., Montreal, Canada). *J. Cell. Biol.* 64(1):89-97; 1975.

DNA fiber autoradiography was used to study the regulation of DNA replication over small lengths of the mammalian chromosome involving one or several replication units (U). DNA fiber autoradiographs were prepared from mouse L-929 cells pulse-labeled with (^{3}H)-thymidine. Initiation events and subsequent chain growth over stretches up to three replication U in length were then studied. It was found that adjacent U generally initiated replication synchronously, this synchrony being related to the proximity of the initiation sites. Synchrony was not absolute; in 1/5 of the pairs, initiation clearly occurred at different points in time. The results also indicated that adjacent U were of similar size and that there was a tendency for neighboring forks, either sharing the same replication U or having adjacent U, to travel at the same rate. Replication U of greater size appeared to have faster rates of chain growth, and there was no evidence obtained for fixed termination sites for DNA replication. These findings suggest that, despite large variations in the size of the replication U, the timing of initiation events, and the rates of fork progression found in chromosomal DNA as a whole, these processes are closely regulated within subchromosomal clusters of active replication units.

0551 RELEASE OF TEMPLATE RESTRICTION FOR DNA SYNTHESIS BY POLY ADP(RIBOSE) POLYMERASE DURING THE HELA CELL CYCLE. (Eng.) Smulson, M. (Georgetown Univ., Sch. Med. Dent., Washington, D. C.); Stark, P.; Gazzoli, M.; Roberts, J. *Exp. Cell Res.* 90(1):175-182; 1975.

The effect of poly ADP-ribose on the release of template restriction during various stages of the HeLa cell cycle was studied. A suspension culture of HeLa cells was synchronized at the G1-S boundary with methotrexate (0.23 µg/ml). After 16 hr, the block was reversed by addition of 10^{-5}M thymidine. The cell number and the rate of DNA synthesis was determined by pulse labeling 3 ml of culture for 10 min with ^{3}H - thymidine at various time intervals. The degree of complexing between DNA and chromosomal proteins and the ability of poly ADP-ribosylation of nuclear proteins to release this template restriction and expose DNA primer sites changed during the HeLa cell cycle. Primer site exposure by NAD and poly ADP-ribose polymerase was assessed with intact nuclei by single deoxynucleotide incorporation into DNA in the presence of saturating bacterial DNA polymerase. The most marked *in vitro* enhancement of primer site exposure by ADP-ribosylation occurred in early G 1 phase, where cellular template restriction was the greatest. Cytoplasmic DNA polymerase also had high activity in early G 1 phase of the cell cycle. Stre-

ptozotocin reduced NAD pools in HeLa cells; a concomitant stimulation of nuclear poly ADP-ribose polymerase activity was noted. It is claimed that it is too early to speculate whether poly ADP-ribose polymerase functions in the intact cell to expose sites for attachment of DNA polymerase.

0552 RNA-PRIMED DNA SYNTHESIS: SPECIFIC CATALYSIS BY HeLa CELL DNA POLYMERASE α.
(Eng.) Spadari, S. (Roche Inst. Mol. Biol., Nutley, N. J.); Weissbach, A. *Proc. Natl. Acad. Sci. U.S.A.* 72(2):503-507; 1975.

Three mammalian DNA polymerases (α, β, γ) were examined to determine whether they would be operable if RNA synthesis by *E. Coli* DNA-dependent RNA polymerase (coupled reaction) were allowed on a DNA strand or if previously synthesized RNA strands still hybridized to HeLa single strand DNA were used as a template (uncoupled reaction). All polymerases were free of RNase H or of deoxyribosenuclease activity. A DNA-RNA hybrid product (uncoupled reaction) was prepared and banded in a CsCl gradient at the density of a single strand DNA; the coupled RNA-primed DNA synthesis is the same as the former but containing deoxyribonucleoside triphosphate and DNA polymerase from HeLa cells. In coupled reactions, DNA polymerases β and γ showed little copying of DNA and were not stimulated by RNA synthesis; but DNA polymerase α was active. This would indicate that the stimulation of DNA synthesis required RNA synthesis. The same results were obtained using an RNA-DNA hybrid and suggest that an RNA of a certain length is necessary to prime DNA synthesis. CsCl-$CsSO_4$ equilibrium centrifugation and ^{32}P transfer experiments showed a covalent association between RNA and DNA, and phosphodiester link from their 3'OH to their 5'OH group, showing a nonrandom distribution of nucleotide sequences. Marked nucleotides, ^{32}P-RNA and ^{3}H-DNA helped determine the length of the DNA molecules formed (100 deoxyribonucleotides linked to an RNA of the same length). The RNA-DNA linked molecule base paired to an HeLa DNA template strand represents a possible natural *in vivo* template for DNA polymerases (α, β, and γ). DNA polymerases β and γ are capable of DNA-, but not RNA-primed DNA synthesis while DNA polymerase α is capable of both RNA- and DNA-primed synthesis.

0553 STUDIES ON PLASMA MEMBRANES. XXII. FATTY ACID PROFILES OF LIPID CLASSES IN PLASMA MEMBRANES OF RAT AND MOUSE LIVERS AND HEPATOMAS.
(Eng.) Van Hoeven, R. P. (Netherlands Cancer Inst., Amsterdam); Emmelot, P.; Krol, J. H.; Oomen-Meulemans, E. P. M. *Biochim. Biophys. Acta* 380(1):1-11; 1975.

The fatty acid patterns of lipid classes in plasma membranes isolated from a transplanted rat hepatoma, two transplanted mouse hepatomas, and two spontaneous mouse hepatomas were compared with those of plasma membranes from normal rat (R-Amsterdam) and mouse (CBA) liver. The lipid classes were phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, lysophosphatidylcholine, free fatty acids, and triglycerides. In rat and mouse liver plasma membranes, these classes demonstrated more or less specific fatty acid profiles. The number of double bonds increased in the order: phosphatidylserine $\geqslant$ phosphatidylethanolamine $>$ phosphatidylinositol $>$ phosphatidylcholine $\gg$ sphingomyelin and lysophosphatidylcholine. Small species differences were noted in most lipid classes. A marked sex difference was observed in the sphingomyelin of mouse liver membranes but not in the phospholipids of rat liver membranes. Fatty acid profiles of mouse hepatoma membranes deviated much less from those of normal mouse liver than did the pattern of the rat hepatoma *versus* rat liver. Cholesterol content was higher in hepatoma membranes than in normal membranes, and in the rat hepatoma this increase was accompanied by a decrease of fatty acyl poly-unsaturation in most lipid classes. A similar decrease in unsaturation was not found for mouse hepatoma plasma membranes. Thus increased phospholipid saturation is not a general feature of neoplastic plasma membranes, nor is an increased cholesterol content of a plasma membrane automatically accompanied by a decrease in fatty acyl unsaturation. This suggests that certain fluctuations in these fluidity parameters are compatible with membrane function and that only particular cell phenotypes require an adjustment to function properly. Free cell surface, extensive in the anaplastic rat hepatoma, could be one of the factors necessitating such an adjustment.

0554 ALTERATIONS IN RIBONUCLEASE ACTIVITIES IN THE PLASMA, SPLEEN, AND THYMUS OF TUMOR-BEARING MICE. (Eng.) Drake, W. P. (Baltimore Cancer Res. Cent., Md.); Kopyta, L. P.; Levy, C. C.; Mardiney, M. R., Jr. *Cancer Res.* 35(2):322-324; 1975.

Six transplantable murine tumor models were evaluated for changes in RNase activity in the lymphoid tissues (spleen and thymus homogenates) of tumor-bearing mice; plasma nuclease levels were also assessed to determine whether or not any changes observed would be reflected in an easily obtainable blood fraction. BW10232, a spontaneous adenocarcinoma, and C1498, a spontaneous myeloid leukemia, were carried in C57BL/6 mice. Harding-Passey, a spontaneous melanoma, and Sarcoma 80, a spontaneous pleomorphic cell sarcoma, were carried in BALB/c mice. C3HBA, a spontaneous adenocarcinoma, and 6C3HED, an induced lymphosarcoma, were carried in C3H/HeJ mice. Tumor lines were transplanted by s.c. injection of a cell preparation obtained by mincing tumor growths. Nuclease activity directed against the synthetic substrates, polyadenylic acid, polyuridylic acid, polyguanylic acid, and polycytidylic acid, was measured and the data obtained for the tumor-bearing animals compared to their normal counterparts. Elevated activity against polyuridylic acid was observed in the plasma of all tumor-bearing mice. Such increases were generally comparable to the increases in activity in the spleen against polyuridylic acid, except that the depression of spleen-associated nuclease activity for C3HBA adenocarcinoma and the insignificant increase in spleen-associated activity for the Sarcoma 180 cell sarcoma broke the pattern. No activity against polyguanylic acid was observed in any of the preparations,

and the degradation of polyadenylic acid was negligible. The observance of unilateral changes in nuclease activity directed against the synthetic substrates demonstrated that, in most cases, two or more enzymes were being detected. Although it was hoped that some of the changes in nuclease activity observed for the lymphoid organs might be reflected in the plasma, no correlation was observed. Because the RNase activity directed against polyuridylic acid was elevated in the plasma of all tumor-bearing mice tested, this type of approach may prove to be a helpful clinical assay in some circumstances or a useful device for the monitoring of the progression of tumor during therapy.

0555 DEOXYRIBONUCLEIC ACID-DEPENDENT RIBONUCLEIC ACID POLYMERASES FROM NORMAL AND POLYOMA-TRANSFORMED BHK-21/C13 CELLS. (Eng.) Cooper, R. J. (Dep. Biochem., Univ. Aberdeen, Scotland); Keir, H. M. *Biochem. J.* 145(3):509-516; 1975.

DNA-dependent RNA polymerase activities in fractions of baby-hamster kidney cells (BHK-21/C13) cells transformed by polyoma virus (PYY cells) were measured to determine if multiple forms of the enzyme exist. DNA-dependent RNA polymerase activities were extracted from the nuclei of BHK-21/C13 and PYY cells, separated on a DEAE Sephadex A-25 column, and then characterized with respect to ionic strength optima, Mn^{2+}/Mg^{2+} activity ratio, and inhibition by actinomycin D, rifampicin and α-amanitin. The A (resistant to inhibition by α-amanitin) and B (sensitive to inhibition by α-amanitin) forms from both cells were similar with respect to $(NH_4)_2SO_4$ ionic strength optima (12.5 mM and 100 mM, resp.). The yields of polymerase relative to DNA content were similar in BHK-21/C13 and PYY cells. The Mn^{2+}/Mg^{2+} ratios for polymerase A from BHK-21/C13 and PYY cells were 1.48 and 0.55, resp., and those of polymerase B were 10.11 for BHK-21/C13 cells and 22.75 for PYY cells. Both A and B forms from both cell types were inhibited by actinomycin D and less so by rifampicin AF/103. X-Amanitin inhibited the B forms from both cell types, but not the A forms. It is concluded that both BHK-21/C13 and PYY cells contain at least two RNA polymerase activities and that the properties of the enzymes are similar to those of RNA polymerases of other eukaryotic systems.

0556 ON THE DNA POLYMERASE III OF MOUSE MYELOMA: PARTIAL PURIFICATION AND CHARACTERIZATION. (Eng.) Matsukage, A. (Natl. Cancer Inst., Bethesda, Md.); Bohn, E. W.; Wilson, S. H. *Biochemistry* 14(5): 1006-1020; 1975.

A high molecular weight membrane-bound DNA polymerase, designated DNA polymerase III, was purified from the mouse myeloma, MOPC-104E, and characterized with respect to physical and reaction properties. DNA polymerase III in whole homogenates from MOPC-104E was solubilized and then purified using a series of ion-exchange chromatographic procedures followed by DNA-cellulose chromatography and glycerol gradient centrifugation. DNA polymerase activity was measured by determining the rate of conversion of ^3H-labeled deoxynucleoside 5'-triphosphate into cold 10% trichloroacetic acid-insoluble material as a result of incubation with enzyme at 37 C for 60 min, using poly(rA)·$(dT)_{12-18}$ as template-primer and Mn^{2+} as divalent cation. Under these conditions, the enzyme was purified as much as 18,000-fold. In the final stages of purification, DNA polymerase III possessed no detectable RNA polymerase activity, nucleoside diphosphokinase activity, or nuclease activity toward DNA or single- and double-stranded RNA. The isoelectric point of the purified DNA polymerase III was approximately pH 5.8; the approximate molecular weight under nondissociating conditions was 315,000 by gel filtration, 270,000 by gel electrophoresis, and 230,000 by sedimentation velocity. The enzyme was not dissociated into smaller species possessing activity by treatment with either 125 mM ammonium sulfate, RNase and DNase, or 500 mM KCl and 0.2% Tween-80. In solutions of relatively low ionic strength, the enzyme aggregated into a much higher molecular weight species. Activity of DNA polymerase III was inhibited by sulfhydryl-blocking reagents and by 1,10-phenanthroline; it was not inhibited by antiserum directed against murine leukemia virus DNA polymerase. DNA polymerase III required a base pair complementary combination of template, primer, and deoxynucleoside 5'-triphosphate for activity. The template specificity varied depending upon whether Mn^{2+} or Mg^{2+} was the divalent cation. Several properties of the polymerase activity such as pH optima, activation energy, and so on were different in reactions with poly(rA)·$(dT)_{12-18}$ and Mn^{2+} as divalent cation than in reactions with poly(A)·$(dT)_{12-18}$ and Mg^{2+} as divalent cation. This suggests that the precise nature of the enzyme reaction under the two conditions is not identical, but the active sites appear to reside in the same protein complex. An enzyme that appears analogous to the myeloma DNA polymerase III was found in normal adult BALB/c mouse liver.

0557 INVOLVEMENT OF TYROSINASE IN MELANIN FORMATION IN MURINE MELANOMA. (Eng.) Hearing, V. J. (Natl. Cancer Inst., Bethesda, Md.); Ekel, T. M. *J. Invest. Dermatol.* 64(2):80-167; 1975.

The possibility that peroxidase is functional in melanogenesis in the murine S-91 melanoma was investigated. S-91 melanomas, passed in CDF_1 mice, were excised (1-2 cm diameter) into 0.1 M phosphate buffer at 4 C and homogenized. Two enzyme fractions were isolated from the homogenate: the melanosomal fraction and the microsomal:soluble fraction. A 6-fold purification of enzymatic activity in the homogenate was achieved by acetone extraction and subsequent dialysis against sodium barbital buffer, with approximately 16% recovery. When this enzyme preparation was applied to a DEAE-cellulose column, 55% of the activity was recovered in the void volume. Tyrosinase was identified as the enzyme responsible for the bulk of melanin formation in the malignant melanocyte. Tyrosinase was capable of utilizing tyrosine as a substrate, as well as dopa, although kinetic measurements indicated that the V_{max} with dopa was much higher than with tyrosine. The affinity of the enzyme for tyrosine is higher than for dopa, and it is suggested that this relationship may in part be responsible

for the occasional misinterpretation of the functional capability of this enzyme. The concept of different tyrosinase isomers being located in different subcellular fractions has been advanced recently and these data seem to support such a theory.

0558 LACTIC DEHYDROGENASE ISOENZYMES IN HUMAN BLADDER CANCER. (Eng.) Bredin, H. C. (Massachusetts Gen. Hosp., Boston); Daly, J. J.; Prout, G. R., Jr. *J. Urol.* 113(4):487-490; 1975.

Lactic dehydrogenase (LDH) isoenzyme levels in normal and neoplastic human urothelium were studied to determine if (1) alterations developed in the course of malignant transformation and if (2) any such alterations correlated with the degree of morphologic differentiation. The histological findings were correlated well with the arithmetic mean value of LDH-I percentage, LDH-V percentage and the LDH-V/LDH-I ratio (LDH-V/I). There was an increase in LDH-V/I in atypical urothelium, an increase in LDH-I percentage in low grade bladder carcinoma and an increase in LDH-V percentage. In high grade bladder carcinoma LDH-V/I increased with a decrease in the LDH-I percentage. Atypical urothelium exhibited significant increase in LDH-V/I with values greater than one typical in high grade, high stage bladder tumors. Since the LDH-V/I in atypical urothelium is significantly altered compared to normal tissue it was postulated that changes in the isoenzyme profile can occur early in the course of neoplastic development. These findings do not represent altered genetic expression with activation of the gene locus controlling M subunit synthesis.

0559 ALTERATIONS IN GLYCOSYLTRANSFERASE ACTIVITY IN HUMAN COLON CANCER. *(Eng.)* LaMont, J. T. (Harvard Med. Sch., Boston, Mass.); Isselbacher, K. J. *J. Natl. Cancer Inst.* 54(1):53-56; 1975.

Five glycoprotein glycosyltransferase enzymes in homogenates of normal and malignant human colon epithelium from the same individual were compared. These enzymes transfer monosaccarides either to partially completed glycoproteins on the cell membrane (endogenous acceptors) or to added glycoproteins or monosaccharide substrates (exogenous acceptors). The levels for exogenous galactosyltransferase and fucosyltransferase and endogenous *N*-acetylglucosaminyl-transferase were higher in the normal tissue. The levels for exogenous and endogenous sialyltransferase and endogenous galactosyltransferase and fucosyltransferase were comparable in the homogenates of normal and cancer cells. Incorporation of fucose and galactose into purified carcinoembryonic antigen (CEA), used as an exogenous acceptor by colon glycosyltransferases, was demonstrated by immunoprecipitation with rabbit antiserum to human CEA. The normal fucosyltransferase and galactosyltransferase showed higher activity with CEA than did the tumor enzymes. It is suggested that reduction of normal tissue antigens on tumor cells may be related to a deficiency of glycosyltransferase enzyme activity. That the appearance of CEA in colon cancer may be the result of such enzyme deficiencies is supported by the observation that galactosyltransferase and fucosyltransferase from normal colon epithelium are more active with CEA as substrate than are cancer transferases.

0560 CORRELATION OF PHENYLALANINE HYDROXYLASE ACTIVITY WITH CELL DENSITY IN CULTURED HEPATOMA CELLS. (Eng.) McClure, D. (M. S. Hershey Med. Cent., Pennsylvania State Univ., Hershey); Miller, M.; Shiman, R. *Exp. Cell Res.* 90(1):31-39; 1975.

Specific activities of phenylalanine hydroxylase and lactic hydrogenase were studied in Reuber hepatoma (H4) cells growing in monolayer culture. Phenylalanine dehydroxylase activity was characterized by a sharp drop upon subculturing to a low cell density (1.8×10^5 cells/cm^2 to 0.25×10^5 cells/cm^2), followed by a period of low and relatively constant specific activity, and finally by a rise to a plateau of high specific activity. This rise coincided with the formation of confluent monolayers (days 2-3). Experiments in which equal numbers of cells were seeded into 100, 60, and 35 mm diameter culture dishes demonstrated that phenylalanine hydroxylase activity was regulated by the population density of the cells in the culture dishes. Neither conditioning of the growth medium, the rate of cell division, nor enzyme leakage from cells appeared to play a major role in the changes observed. Lactic dehydrogenase activity showed a much smaller but still cell density-dependent variation. Although the mechanisms of regulation of phenylalanine are not known, the enzyme appears to be a very sensitime indicator of some type of cell-cell interaction.

0561 CARBAMYL PHOSPHATE SYNTHETASES IN RAT LIVER NEOPLASMS. (Eng.) Lawson, D. (Temple Univ. Sch. Med., Philadelphia, Pa.); Paik, W. K.; Morris, H. P.; Weinhouse, S. *Cancer Res.* 35(1):156-163; 1975.

Variations in the activity of the isozymes of carbamyl phosphate synthetase (CPS), CPS I (a mitochondrial enzyme found exclusively in the liver, which is involved in urea synthesis), and of CPS II (a soluble cytoplasmic enzyme widely distributed in animal tissues), were studied in normal rat liver and in Morris hepatomas. The isozyme levels in the liver and tumor tissues were assayed using colorimetric and radiochemical methods. CPS I was absent from the fast-growing, poorly-differentiated hepatomas (Novikoff hepatoma and Morris hepatomas 3924A and 9098F), but was present in the more slowly growing, well- and highly-differentiated Morris hepatomas. The CPS I activity did not, however, correlate closely with the growth rate or degree of differentiation; activity was high in normal liver and the slowly growing 21, 47C and 28A hepatomas, but was very low in other slow-growing, highly-differentiated hepatomas (9618A, 66 and 16). CPS II showed low activity in the normal liver samples and in all hepatomas examined, the activity being lowest in the fast-growing, poorly-differentiated hepatomas. There was

a substantial lowering of the CPS I activity in the livers of rats bearing large, slow-growing tumors with high CPS I activity. As the tumors grew in size and the liver CPS I activity decreased, a relatively constant total CPS I activity was maintained. This effect, which was not observed in rats bearing fast-growing hepatomas or slow-growing hepatomas with low CPS I activity, was not due to specific nutritional effects of the tumor on the host. The phenomenon suggests the presence of a homeostatic mechanism operating at the level of the whole animal, and further suggests that the level of CPS I may have an important bearing on nitrogen metabolism.

0562 GLUCOCORTICOID RECEPTORS IN MOUSE MAMMARY TUMORS: SPECIFIC BINDING TO NUCLEAR COMPONENTS. (Eng.) Shyamala, G. (Cancer Res. Lab., Univ. California, Berkeley). *Biochemistry* 14(2): 437-444; 1975.

The specific interactions of glucocorticoids in mouse mammary tumor nuclei were studied. Mammary tumor slices from female GR mice were incubated *in vitro* with (^{3}H)dexamethasone at 25 C; known concentrations of various radioactive and nonradioactive steroids were added in some cases. The uptake and distribution of dexamethasone in the slices were studied by Sephadex G-25 filtration, sucrose gradient techniques, and liquid scintillation counting. The mammary tumor slices were found to contain a limited number of specific nuclear binding sites, which were saturated with dexamethasone concentrations of 10^{-8} M. The number of specific binding sites in the tumor nuclei varied between individual tumors and was related to the concentration of cytoplasmic binding sites in the unincubated tissue. The binding component in the nuclei, which was easily solubilized with 0.4 M KCl-containing buffers, appeared to be a protein. The ability of various corticoids to compete for the (^{3}H)dexamethasone binding sites *in vitro* was correlated with their glucocorticoid potency. Estradiol and progesterone (10^{-6} M) were also effective competitors for the glucocorticoid binding sites. However, unlike the glucocorticoids, such as hydrocortisone and corticosterone, which translocated to nuclear sites which were also specific for dexamethasone, estradiol and progesterone competed for the cytoplasmic binding sites and did not translocate to the nucleus. These and other data suggest that high concentrations of estrogen and progesterone may inhibit the binding of glucocorticoids to their receptor sites in mammary tumor cells, thus counteracting the growth-promoting effect of the glucocorticoids.

0563 MEASUREMENT OF INITIAL PROSTAGLANDIN F METABOLITES IN MEDIUM OF BALB/c 3T3 AND SV3T3 MOUSE FIBROBLAST CULTURES. (Eng.) Ritzi, E. M. (Worcester Found. Exp. Biol., Shrewsbury, Mass.); Boto, W. O.; Stylos, W. A. *Biochem. Biophys. Res. Commun.* 63(1):179-186; 1975.

The levels of the initial metabolites of prostaglandins F_1 and F_2 were measured in BALB/c 3T3 and SV3T3 mouse fibroblast cultures to determine whether metabolism differences could account for previously observed differences in the concentrations of primary prostaglandins in these culture media. Medium levels of 13, 14,dihydro-15-keto-prostaglandin F_1 and 9α, 11α, 15-trihydroxy prostanoic acid (F_{0_α}) were measured during radioimmunoassay. The prostaglandin metabolite levels were proportional in both cell lines and were 5-17% of those of the primary prostaglandins present. It is suggested that the metabolism of prostaglandins in these cultures is slow and that differences in prostaglandin F concentrations in the culture medium may arise from differences in synthesis or release by the cells.

0564 NUCLEAR MAGNETIC RESONANCE INVESTIGATIONS OF HUMAN NEOPLASTIC AND ABNORMAL NONNEOPLASTIC TISSUES. (Eng.) Eggleston, J. C. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.); Saryan, L. A.; Hollis, D. P. *Cancer Res.* 35(5):1326-1332; 1975.

The first study to compare the nuclear magnetic resonance (NMR) relaxation time of benign neoplastic, nonneoplastic abnormal tissues and human cancer tissue was undertaken and the use of this technique in discriminating cancer from other abnormalities was evaluated. A total of 279 tissue samples were obtained from 118 patients from areas involved by the disease in question. The spin-lattice magnetic relaxation time (T_1) was determined for all tissues. In breast tissue the T_1 values were scattered throughout the range of all samples with the lowest reading occurring where high fat content was evident. Uninvolved areas of the intestinal tract had longer T_1 values than actual tumor tissue. No discrimination could be made between prostatic hyperplasia and carcinoma using T_1 values. T_1 values in other soft tissue were inconclusive. The relationship between T_1 values of cancer and other tissue thus did not permit recognition of cancer in this manner. This discrepancy is due to noncancerous-specific changes in water and fat content and also to the extreme variation in tissue components in malignant tissue. The NMR technique does not provide a valuable primary or adjunct method of cancer diagnosis.

0565 ELECTRON SPIN RESONANCE FOR DETECTING POLYADENYLATE TRACTS IN RNA'S. (Eng.) Bobst, A. M. (Dep. Chem., Univ. Cincinnati, Ohio); Sinha, T. K.; Pan, Y. C. E. *Science* 188(4184):153-154; 1975.

A novel assay for the detection and quantitation of poly(A) tracts in RNA systems is reported. It is based on monitoring the complex formation between spin-labeled polyuridylate [poly(U)] or poly (2'-deoxy-2'-fluoro) uridylic acid [poly(dUfl)] and the poly(A) tail by electron spin resonance spectroscopy (ESR); the advantages reported include rapidity and directness. High molecular weight fractions of poly(U) and poly(dUfl), with a ratio of spin label to nucleotide of 1:75, were used. The effect of hybridization of spin-labeled poly(U) with poly(A) on the ESR line shape was shown to decrease the mobility of the spin label upon complex formation.

Raising the temperature increased the mobility at the spin denaturation temperature, reflecting the temperature dependent transition from double strands to single strands. The spin-labeled complex probe is also subject to an anisotropic motion characteristic of the complex formed. The formation of double-stranded spin-labeled poly(U) x poly(A) or poly(dUfl) x poly(A) was also directly monitored by ESR spectroscopy by addition of a small known amount of poly(A) to a known amount of poly(U) or poly(dUfl). For both, the ratio of the high field:center field hyperfine constants remained constant as soon as both components of the hybridization product were present in equal amounts; a two-segment titration plot was formed. Trapping a poly(A) tail in a viral or eukaryotic mRNA required the predetermination that the tail is freely accessible for hybridization with poly(U) or poly(dUfl). Stacked adenylate segments not base-paired, of an average length of 40-60 nucleotides, were directly observable by circular dichroism spectroscopy in rabbit globin mRNA. It was subsequently shown by ESR spectroscopy that the presence of this poly(A) tail could be qualitatively determined with spin-labeled poly(U) and poly(dUfl). Comparison of the values obtained illustrated that slightly less of the poly(A) segment as apparently seen by CD spectroscopy is accessible to hybridization with spin-labeled poly(dUfl). It is emphasized that the novel spin-label assay requires a maximum of 36 μg of poly(A)-containing RNA, and has potential for direct quantitation of RNA tumor viruses.

0566 TISSUE WATER CONTENT AND NUCLEAR MAGNETIC RESONANCE IN NORMAL AND TUMOR TISSUES. (Eng.) Kiricuta, I.-C., Jr. (Biophysical Department, Oncological Institute of Cluj, Str. Republicii nr. 34-36, Cluj, Romania); Simplaceanu, V. *Cancer Res.* 35(5):1164-1167; 1975.

Pulsed proton nuclear magnetic resonance (NMR) was used to differentiate between normal and malignant tissues. Mature Wistar rats with the tumors Walker 256 carcinoma, epithelioma T8 Guerin, H-18R, and R20 along with eight- to ten-week-old RAP mice bearing Ehrlich-Lettre tetraploid ascites and Ehrlich solid carcinoma were used. Spin-lattice (T_1), spin-spin (T_2) relaxation times and tissue water content were measured. There was a significant increase in the T_1 and T_2 relaxation times and in percentage water content in all tumor tissues. The increase of free water showed a good correlation with observed T_1 values. The T_1 and T_2 values in immature brain and heart tissue had the same magnitude as that of malignant tumors, indicating that changes in relaxation times are not due to the increase in freedom of tissue water molecules or to water structure. There was a good correlation between the increase in relaxation times and increase in water content in immature tissues. The results suggest that the main cause of the observed differences between the relaxation times of normal and malignant tissue is the greater tissue hydration in the neoplasms. The T_1 and T_2 times thus decreased as tissues matured as a result of decreased water content. T_1 is more sensitive than T_2 to variations in tissue water content. The NMR technique appears less promising in distinguishing cancerous tissue if water content is the principal cause of the observed differences between the relaxation times.

0567 ATTEMPTS TO DETECT *AGROBACTERIUM TUMEFACIENS* AND BACTERIOPHAGE PS8 DNA IN CROWN GALL TUMORS BY DNA·DNA-FILTER HYBRIDIZATION. (Eng.) Farrand, S. K. (Dept. Microbiology, Univ. Washington, Seattle, Wash. 98195); Eden, F. C.; Chilton, M.-D. *Biochim. Biophys. Acta* 390(3):264-275; 1975.

A systematic study of the DNA·DNA filter reaction is presented which measures its ability to detect small amounts of simple DNA (bacterial or bacteriophage) in model mixtures of DNA immobilized on filters. Saturation curves show qualitatively that significant binding occurs where there is 10% *Agrobacterium tumefaciens* DNA on the filter but not 1%. PS8 bacteriophage DNA is detectable at a level of 0.1%. True saturation is not attained in the bacterial DNA reaction: radioactivity bound represents only 3% of the theoretical saturation value. The bacteriophage DNA reactions attain 15-30% of the expected saturation value. When crown gall tumor DNA filters were tested for the presence of *A. tumefaciens* or PS8 bacteriophage DNA by saturation reactions, an apparently significant amount of binding was observed compared with usual background levels for heterologous DNA filters. However thermal dissociation profiles revealed that no well-matched duplexes were formed. Normal tobacco callus DNA filters exhibited the same type of binding of labeled DNA to a similar extent (50-100% as much as tumor DNA filters). Both types of DNA-filters bound *Bacillus subtilis* and bacteriophage T4 DNA as efficiently as *A. tumefaciens* and PS8 DNA. The high non-specific background binding of labeled DNA by filters containing DNA isolated from plant tissue culture materials is ascribed to low single strand molecular weight of the filterbound DNA. This study provides no evidence for foreign DNA in crown gall tumors, and raises objections to the interpretation of the data of earlier investigators who claimed to detect *A. tumefaciens* DNA in crown gall tumors by DNA·DNA-filter hybridization.

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LANGUAGE ABBREVIATIONS

Afr.	Afrikaans
Ara.	Arabic
Bul.	Bulgarian
Chi.	Chinese
Cro.	Croation
Cze.	Czech
Dan.	Danish
Dut.	Dutch
Eng.	English
Est.	Estonian
Fin.	Finnish
Fle.	Flemish
Fre.	French
Geo.	Georgian
Ger.	German
Gre.	Greek
Heb.	Hebrew
Hun.	Hungarian
Ice.	Icelandic
Ind.	Indonesian
Ita.	Italian
Jpn.	Japanese
Kor.	Korean
Lav.	Latvian
Lit.	Lithuanian
Nor.	Norwegian
Pol.	Polish
Por.	Portuguese
Rum.	Rumanian
Rus.	Russian
Ser.	Serbo-Croatian
Slo.	Slovak
Spa.	Spanish
Swe.	Swedish
Tha.	Thai
Tur.	Turkish
Ukr.	Ukrainian
Vie.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

A	angstrom(s)
ACTH	adrenocorticotropic hormone
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BCG	Bacillus Calmette Guerin
bid	twice daily
C	degrees centigrade
cal	calorie(s)
kcal	kilocalorie(s)
cc	cubic centimeter(s)
Ci	curie(s)
mCi	millicurie(s)
μCi	microcurie(s)
cm	centimeter(s)
CNS	central nervous system
cpm	counts per minute
dl	deciliter(s)
ml	milliliter(s)
μl	microliter(s)
DNA	deoxyribonucleic acid
ED_{50}	median effective dose
EDTA	ethylenediamine tetraacetic acid
ESR	erythrocyte sedimentation rate
g	gram(s)
kg	kilogram(s)
mg	milligram(s)
μg	microgram(s)
Hb	hemoglobin
hr	hour(s)
ia	intra-arterial
ic	intracerebral
icav	intracavitary
id	intradermal
ILS	increased life span
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratumorous
IU	International Unit
iv	intravenous
K_m	Michaelis constant
LD	lethal dose
LD_{50}	median lethal dose
m	meter(s)
mm	millimeter(s)
M	molar
mM	millimolar
μM	micromolar
mOsm	milliosmolar
mEq	milliequivalents
min	minute(s)
mo	month(s)
MTD	maximum tolerated dose
N	normal concentration
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotidephosphate
NADPH	reduced nicotinamide adenine dinucleotide-phosphate
ng	nanogram(s) (10^{-9})
od	once daily
Pa	ambient pressure
PAS	periodic acid-Schiff
pg	picogram(s) (10^{-12})
pgEq	picogram equivalent
po	orally
ppb	parts per billion
ppm	parts per million
qid	four times daily
qod	every other day
QO_2	oxygen quotient
R	roentgen(s)
RBC	red blood cells (erythrocytes)
RNA	ribonucleic acid
sc	subcutaneous
sec	second(s)
SGOT	serum glutamic-oxalacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SRBS	sheep red blood cells
TCD	tissue culture dose
TCD_{50}	median tissue culture dose
tid	three times daily
U	unit(s)
mU	milliunit(s)
UV	ultraviolet
vol	volume
WBC	white blood cells (leukocytes)
wk	week(s)
wt	weight
x	times
yr	year(s)

CONTENTS

0601 PROLACTIN AND BREAST CARCINOMA. (Eng.) Smithline, F. (Queens Hosp. Cent., Jamaica, N. Y.); Sherman, L.; Kolodny, H. D. *N. Eng. J. Med.* 292(15):784-792; 1975.

Research on the possible relation of prolactin to human breast carcinoma is reviewed. The secretion of prolactin by the anterior pituitary in man and other mammals is under dual control of hypothalamic neurohormones that act as releasing and inhibiting factors. Furthermore, certain pharmacologic agents are found to affect the synthesis, release, or action of prolactin-inhibiting factors or to directly affect prolactin secretion. A multiplicity of hormones are necessary for mammary growth and development in mice; it is implied that the mechanism of prolactin action is not dependent on cell penetration, but involves surface membrane receptors. Interspecies comparisons include considerations of species genetics, tumor characteristics, prolactin effects, and etiologic factors. Early first pregnancy in women is associated with decreased risk of breast carcinoma, possibly due to the resulting increased levels of hormones prior to the biochemical transformation of human breast tissue, which serves in "protecting" the breast. Later first pregnancies may be capable of stimulating cells that are already biochemically transformed, and hence associated with increased risk. In female Sprague-Dawley rats, development of hyperplastic nodules and their progression to mammary carcinoma are at least in part dependent on prolactin; the lowering of serum prolactin with ergot drugs suppresses the development of nodules, decreases the frequency of mammary tumors, but has little effect on established tumors. Paradoxically, it is found that both increases and decreases in prolactin inhibit the induction of mammary tumors by dimethylbenzanthracene. Whereas the role of prolactin is less clear in human breast cancer than in the laboratory rodents, prolactin is suggested as a factor in certain high risk populations. Some, but not all tumors appear to be prolactin-dependent for continuous viability or growth. The results of treatment with L-dopa remain ambiguous and diversified. Furthermore, environmental factors, including a high fat diet, are associated with increased prevalence of mammary carcinoma. The ability of prolactin to stimulate mammary-tumor growth may depend less on serum levels of the hormone than on the number of receptor sites in the tumor tissue that are able to react with the hormone. (92 references)

0602 COVALENT INTERACTION OF CARCINOGENS WITH DNA. (Eng.) Brooks, P. (Inst. Cancer Res., London, England). *Life Sci.* 16(3):331-344; 1975.

Theories of chemical carcinogenesis based on the concept that such carcinogenesis is mediated *via* a reactive electrophilic intermediate are reviewed. The suggested relation of carcinogenesis to mutagenesis, and the subsequent somatic mutation theory, implies that cancer is a consequence of DNA modification. This theory was periodically overshadowed by the protein deletion theory of cancer, which arose from the demonstrated binding of carcinogens to soluble proteins. However, covalent binding of hydrocarbons to highly purified DNA, as well as to RNA and protein, is found; furthermore, there is some degree of correlation between the carcinogenic potency of a series of hydrocarbons and their extent of mouse skin DNA binding. The mechanism by which aromatic amines are metabolically converted into electrophilic species is elucidated; esterification of the *N*-hydroxyl group yields a highly reactive derivative capable of modifying DNA, RNA, and protein. The classical alkylating agents are shown to react with the ring nitrogen atoms of the nucleic acid bases, in particular with the N-7 position of guanine moieties. The nitrosamides do not require metabolic activation but are activated by anions, yielding simple alkyl carbonium ions. Work in bacteriophage mutagenesis indicates a correlation between the ability of alkylating species to induce tumors and the extent to which they alkylate the O-6 position of guanine in DNA, suggesting a relationship between carcinogenesis and transition mutation. Potent natural product carcinogens include cycasin and its aglycone, the pyrrolizidine alkaloids, saffrole, and aflatoxin B_1. The unsaturated 2:3-double bond is a necessary structural feature, with a good correlation established between microsomal-dependent DNA binding and carcinogenicity. The relevance of DNA repair to carcinogenesis is just beginning to be considered. (19 references)

0603 ENVIRONMENTAL *N*-NITROSO COMPOUNDS: IMPLICATIONS FOR PUBLIC HEALTH. (Eng.) Wogan, G. N. (Dep. Nutr. Food Sci., Massachusetts Inst. Technol., Cambridge); Tannenbaum, S. R. *Toxicol. Appl. Pharmacol.* 31(3):375-383; 1975.

The existence of *N*-nitroso compounds in the environment is a generic problem that exemplifies the most difficult aspects of foodborn environmental chemical hazards. The number of compounds of interest is potentially large, and either the compounds themselves or reactants capable of forming them are widely distributed in the environment. Both factors create a large potential for exposure, which is probably long-term and low-level in character, a property that makes it difficult to establish cause and effect relationships. All animal species and most animal organs appear to be susceptible to the carcinogenic action of *N*-nitroso compounds. In man, *N*-nitroso compounds are suspected causative agents in the high incidence of esophageal cancer in Iran and parts of Africa; they have also been implicated in the high risk for gastric carcinoma found among Chilean migrants in Colombia. However, field epidemiology is of limited usefulness because of the long latent period and relatively low absolute frequency of the suspected disease in the general population. Chemical experiments have not been helpful in defining the hazards of *N*-nitroso compounds in food supplies because of the semiquantitative character of the experiments, while animal studies have involved the feeding of reactants at levels far in excess of those encountered in food. Assessment of the public health risk must, therefore, be based on further characterization of nitrosating agents, nitrosatable compounds, and reaction conditions under which nitrosation can occur. (55 references)

0604 DRUGS IN CLINICAL USE WHICH CAUSE CANCER. (Eng.) Hoover, R. (Natl. Cancer Inst., Bethesda, Md.); Fraumeni, J. F., Jr. *J. Clin. Pharmacol.* 15(1):16-23; 1975.

The carcinogenicity of drugs in clinical use is examined with respect to those with recognized carcinogenic potential for humans, with carcinogenic potential in laboratory animals but with no demonstrated carcinogenicity in man, and with drugs not yet evaluated in man. Difficulties in evaluating the cancer risk of suspected agents are summarized, and guidelines are presented for selecting drugs for epidemiologic investigations. Drugs associated with human cancer are radioisotopes, immunosuppressive drugs (for renal transplantation), cytotoxic drugs, hormones, and agents such as arsenic and coal-tar ointments. Drugs that are carcinogenic in the laboratory but are not associated with human cancer are isoniazid and female sex hormones, including natural and artificial estrogens and progestin. Future evaluations of these drugs will have to include factors such as age, latent period and cocarcinogen exposures. Drugs that are carcinogenic in animals and which warrant prompt epidemiologic study include chemotherapeutic agents, tertiary amines and iron dextran. Thus, selection of drugs for evaluation in man should be based on information provided by the clinical pharmacologist, as well as on the expertise of laboratory scientists and epidemiologists. (51 references)

0605 IONIZING RADIATION AS A CARCINOGEN: PRACTICAL QUESTIONS AND ACADEMIC PURSUITS. (Eng.) Mole, R. H. (Med. Res. Counc., Oxfordshire, England). *Br. J. Radiol.* 48(567):157-169; 1975.

Ionizing radiation-induced cancer is considered in this review. Enumeration of the number of cases of induced cancer to be expected after a given exposure depends on a set of working hypotheses: 1) no dose of radiation, however small, is without some chance of causing cancer; 2) the increase in cancer with increasing radiation exposure is linearly proportional to the dose of radiation; and 3) whatever the particular circumstances under consideration, the cancer rate/U dose is given directly by human experience. Each practical consideration has to be put in two parts, a scientific part and a non-scientific part (i.e., is the chance of cancer induction by a particular kind and level of radiation exposure acceptable given the purposes for which the exposure is to be incurred?). Central to the question of whether small radiation exposures are carcinogenic is the effect of antenatal radiography. In Japanese bomb survivors, most radiation-induced cancer has been found in those irradiated in adult life, less in those exposed in childhood and adolescence, and least for exposure *in utero*. Specific biological differences between different kinds of malignant disease are becoming increasingly evident in their induction by ionizing radiation. The cancer rate, after a given exposure, can vary greatly in different tissues. When dose-response relationships for observed cancer frequencies are used as evidence for dose-response relationships for cancer induction, it is necessary to allow for concomitant cell sterilization. When this is done, there is little support for linearity as the method of extrapolation when making predictions about the possible effects of low doses. The other two working hypothses, however, seem to be increasingly acceptable.

0606 VIRUSES: CAUSATIVE AGENTS OF CANCER. (Eng.) Sheinin, R. (No affiliation given). *Laryngoscope* 85(3):468-486; 1975.

An overview of the conceptual and practical tools for the detection of tumor viruses is presented, with applications to studies of human malignant disease. Special reference is made to laryngeal cancer. The *in vivo* test in which homologous newborn animals serve as recipients is widely utilized in identification of many naturally occurring tumor viruses. In addition, an *in vitro* test dependent upon the fact that both normal and neoplastic cells can be removed from the animal and grown in culture is much utilized in studying physiological transformations and altered cell morphology. Key observations noted in the study of tumor viruses include the recognition of many different viruses to related members of the same virus group, and the superficially similar physiological cell interaction of oncogenic and nononcogenic viruses. Available evidence indicates a primary event in neoplastic conversion of normal cells is a true genetic transformation; the second step involves expression of the T-gene. Such virogene expression can be induced by a number of physiological factors involved in cell growth and differentiation. This induction may involve either expression of a portion or a whole virogene. Likewise, tools for the study of tumor viruses include both direct and indirect tests. Virus particles present may be theoretically detected by electron microscopy and functional tests; detection when no virus particles are produced may require molecular hybridization and immunochemical procedures. The indication that human tumors are caused by microorganisms is evidenced by the detection of human papilloma virus, human papovavirus, several human adenoviruses, plus the implication of the Epstein-Barr virus and herpes virus Type 2 in neoplastic disease. Evidence available to relate viruses and laryngeal cancer relates to papilloma of the larynx, and to the electron microscopic detection of nuclear inclusions of particles. Chemical studies include the implication of human papilloma virus in transforming newborn laryngeal cells, and a seroepidemiological survey considering herpes viruses in the etiology of laryngeal carcinoma. Whether viruses only represent distracting artifacts, or whether they are true causes of human malignant disease, remains to be determined. (24 references)

0607 VIRUSES ASSOCIATED WITH HUMAN LEUKAEMIA. (Eng.) Weiss, R. (No affiliation given). *Nature* 254(5496):101-102; 1975.

Three recent reports corroborating the association of a C-type virus with human leukemia are reviewed. The appearance of virus-like particles in medium from short-term cultures of leukemia bone

marrow cells from both lymphocytic and myelocytic leukemia patients, but not from cultures of normal individuals, is reported. Furthermore, cultures of peripheral WBC from leukemia patients do not release virus-like particles. Another research group describes the production of virus from cultures of three myeloblastic cell lines from the peripheral blood of a patient with acute myelogenous leukemia, and shows electron micrographs of C-type virus particles, including budding forms. These viruses possess all the physical and chemical properties characteristic of C-type virus particles; in addition, the ability to maintain long-term, exponentially proliferating cultures of myeloblasts was noted. A third study reports antigens related to a major structural protein (p30) of the woolly monkey and gibbon C-type viruses present in the WBC of five acute leukemia patients. A viral entity associated with human leukemia cells has been previously suggested, and a DNA polymerase with the properties of viral reverse transcriptase was regularly detected in human leukemic cells. The p30 proteins are described as indistinguishable in a radioimmune competition assay from the p30 of the woolly monkey virus. Factors discounting the possibility of contaminating animal viruses are discussed, and the suggestion that at least one component of the woolly monkey virus complex was acquired from man is presented. In contrast to feline leukemia, there is no epidemiological evidence that human leukemia is contagious, except for one recent report of possible clusters in incidence of leukemia. (11 references)

0608 TUMOR VIRUSES. (Eng.) Anonymous. *Cold Spring Harbor Symp. Quant. Biol.* 39:1-1200; 1975.

Published in two books, the first dealing with papova- and adenoviruses, the second with herpes- and RNA tumor viruses, this volume consists of 134 separate papers, most of which relate to genetics, transcription, and replication. Other papers cover viral proteins and antigens, expression of endogenous viruses, and effects of transformation.

0609 SURFACE REORGANIZATION AS AN INITIAL INDUCTIVE EVENT IN THE DIFFERENTIATION OF PROTHYMOCYTES TO THYMOCYTES. (Eng.) Boyse, E. A. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Abbott, J. *Fed. Proc.* 34(1):24-27; 1975.

An hypothesis for surface reorganization regulated by the genome in response to the environment as an initial inductive event in the differentiation of prothymocytes to thymocytes in the adult mouse is presented. The T-cell induction assay served as a model for phenotypic regulation during certain types of differentiation. It involves induction and observation of prothymocyte conversion to thymocyte *in vitro*. Conversion includes manifestation of products of at least six gene loci and change in surface markings and antigens. This genetic program is predetermined and present before prothymocytes migrate to thymus. *In vitro*, induction was initiated by many agents. *In vivo*, only a thymic agent is active. Conversion occurs before cell division. It is suggested that this specific thymic agent, a polypeptide, engages a specific receptor on prothymocytes and initiates expression of the thymic phenotype *via* adenylate cyclase and cyclic AMP. This induction system is compared to one which might operate in differentiation-induction processes in embryo. Thus, the assay reveals in the inductive process an essential early state which involves surface reorganization prior to cell division and functional maturation. (25 references)

0610 ROLE OF MACROPHAGES IN IMMUNITY, WITH SPECIAL REFERENCE TO TUMOUR IMMUNOLOGY: A REVIEW. (Eng.) Lejeune, F. J. (Dep. Surg., Inst. Jules Bordet, Brussels, Belgium). *Biomedicine* 22(1): 25-34; 1975.

A survey of the various mechanisms implicated in the role of macrophages in immunity is presented. A discussion of macrophages includes their morphology (with particular mention of the ruffled membrane forming dendritic processes), the analogous capacities to phagocytose and to spread, the trypsin resistance, and the ubiquitous nature of tissue macrophages. It is noted that whereas the bulk of research was done using peritoneal macrophages, the results may not be completely applicable to other macrophages (e.g. alveolar or spleen). The role of macrophages in immunity includes the uptake and "processing" of antigens by macrophages as the first step of the affluent arc of the immune response, plus a major role in the effluent arc. It is suggested that in the immune/reaction, delayed hypersensitivity, specific cytotoxicity, and activation are related to lymphocyte-macrophage cooperation. Evidence for the involvement of macrophages in tumor immunity includes a demonstration of macrophage migration inhibition using tumor membrane antigens. The nature of macrophage cytotoxicity appears to vary according to the experimental system; it can be specific or nonspecific, and it can kill or only inhibit the tumor cell growth. Possible mechanisms of macrophage cytotoxicity include cytophilic antibodies, the Specific Macrophage Cytotoxin, and the Specific Macrophages Arming Factor. Numerous nonspecific mechanisms include the lysosomal system and some variants of the antibody cell-mediated immunity. It is emphasized that immunotherapeutic organisms (e.g. *Bacillus Calmette-Guerin*) can immunostimulate and also immunodepress. Among the various mechanisms involved in the effect of immunotherapy, two major phenomena are: a direct effect on macrophages (especially their lysosomal system), and macrophage-mediated stimulation of T and/or B lymphocytes. (130 references)

0611 ATROPHIC GASTRITIS: STRUCTURAL AND ULTRASTRUCTURAL ALTERATIONS, EXFOLIATIVE CYTOLOGY AND ENZYME CYTOCHEMISTRY AND HISTOCHEMISTRY, PROLIFERATION KINETICS, IMMUNOLOGICAL DERANGEMENTS AND OTHER CAUSES, AND CLINICAL ASSOCIATIONS AND SEQUELLAE. (Eng.) Glass, G. B. J. (New York Med. Coll., N. Y.); Pitchumoni, C. S. *Hum. Pathol.* 6(2):219-250; 1975.

Histological and clinical factors involving atrophic gastritis are reviewed. Histological changes in

chronic gastritis, studied by gastric suction and endoscopic biopsy, involve superficial epithelium, glandular tubules, and lamina propria. Studies of the histology of the main forms of chronic gastritis describe graded forms of chronic superficial gastritis, atrophic gastritis, gastric atrophy, and antral gastritis. Studies of incidence of atrophic gastritis compare a group of "healthy" Finns, and a collection of Australian patients of a more advanced age; the incidence of gastritis in patients with dyspeptic phenomena is very high. Classifications of chronic gastritis have emerged from the three basic forms initially recognized: chronic superficial gastritis, atrophic gastritis, and gastric atrophy; several grades of superficial and atrophic gastritis are distinguished, seven grades of chronic fundal gastritis are mentioned, and differentiated chronic superficial gastritis is noted. In addition to histological classifications based on fundal lesions, a more complete classification is presented involving four elements: (1) the mucosal portion where the lesions are found, (2) the type of gastritis, (3) the activity of gastritis, and (4) the presence or absence of metaplasia and its type. A comparison of the various classifications of abnormal exfoliated or abraded surface epithelial or crypt cells is described, with a good correlation between maturation abnormality as detected by cytological techniques and by measurement of the proliferation kinetics. The enzyme histochemistry and cytochemistry of atrophic gastritis is reviewed, with enzyme cytochemistry noted as superior. Physiological and pathological variations in gastric cell kinetics reveal a kind of dynamic equilibrium, and indicate the existence of an immunological mechanism regulating cell proliferation kinetics of the gastric mucosa. Various mechanisms and causes of atrophic gastritis are presented. Immunological derangements include humoral immunological derangements and cellular hypersensitivity derangements; three significant nonimmunological causes are chronic alcoholism, bile reflux into the stomach, and surgical operations on the stomach, especially subtotal gastrectomy. Gastric ulcer, adenomatous polyps, gastric carcinoma, pernicious anemia, and iron deficiency anemia are the most important clinical conditions accompanying or following atrophic gastritis. (179 references)

0612 CARCINOGENESIS OF LARYNGEAL CARCINOMA. (Eng.) Saffiotti, U. (Natl. Cancer Inst., Bethesda, Md.); Kaufman, D. G. *Laryngoscope* 85(3): 454-467; 1975.

Experimental models for the induction of carcinoma of the larynx and for study of its pathogenesis are reviewed. The Syrian golden hamster has been the experimental species of choice, due to its susceptibility to respiratory carcinogenesis and its resistance to respiratory infection. The main categories of chemical exposures have been shown to be effective in inducing laryngeal tumors. Intratracheal administration of polynuclear hydrocarbons, which includes as its prototype benzo(*a*)pyrene, showed that the respiratory tumor response was related to the size and frequency of carcinogen doses, and the length of time of exposure in the lungs. Illustrations of observed pathologic changes are presented; most tumors were squamous carcinoma with keratinization, frequently with formation of keratin pearls, and located at the vocal chords or in the lower part of the larynx. N-nitroso compounds, i.e. nitrosamines and nitrosamides, had been previously found highly carcinogenic and illustrating species organotropism; a particular organotropism for the respiratory tract of the hamster was observed for diethylnitrosamine. However, in contrast to tumors induced by polynuclear hydrocarbons, they are found histologically benign, usually papillary in structure, and lined with either squamous or columnar mucous epithelium. After successful induction of respiratory tract lesions within hamsters exposed to inhalation of tobacco smoke, the larynx showed extensive and severe alterations including leukoplakias, pseudoepitheliomatous hyperplasia, and *in situ* and invasive carcinomas occurring over a long period of time. Three main lines of research have been developed to investigate the pathogenesis of respiratory tract cancers. These include *in vivo* studies identifying those conditions or factors that enhance or inhibit induction of respiratory cancers, *in vitro* organ or cell culture systems in studying isolated target tissues, and *in vivo* and *in vitro* methods addressed to the identification of key mechanisms. *In vitro* systems for studying the effects of contributory host factors employ both short-term and long-term cultures. The possibility of screening the human population for susceptibility to carcinogenesis and preventive medical measures is suggested. (45 references)

0613 CLUSTERING OF ABERRATIONS TO SPECIFIC CHROMOSOMES IN HUMAN NEOPLASMS. (Eng.) Levan, G. (Inst. Genet., Lund, Sweden); Mitelman, F. *Hereditas* 79(1):156-160; 1975.

The nonrandom chromosome variations in many types of experimental and human tumors is discussed, and it is suggested that specific chromosome constitutions (genotypes), are more favorable for malignant development than others. In chronic myeloid leukemia, the Ph^1 chromosome, identified as a deleted chromosome No. 22, is established as a consistent structural aberration. In acute myeloblastic and lymphoblastic leukemia, frequent involvement of chromosome No. 8 and No. 21 is found; plasma cell leukemia exhibits a chromosome No. 14 with two extra terminal bands. Abnormalities of F group chromosomes, specifically No. 20, are associated with polycythemia vera. In various nonmalignant, premalignant, and malignant hematologic disorders, aneuploidy involving C group chromosomes is the most common chromosome abnormality. In addition to the abnormalities of chromosomes No. 14 and No. 8 associated with Burkitt lymphomas, consistent other abnormalities are found in other malignant lymphomas, meningiomas, and colonic polyps. The nonrandom chromosome variation of such human neoplasms reveals obvious clustering to a few specific chromosomes. The etiologic factor is decisive in the formation of the chromosome pattern; despite differences there are also points of agreement in the chromosome patterns induced by different hydrocarbons. It is suggested that the carcinogenic potency of any given

mutagen parallels the selectivity of concentration of effects to chromosomal gene sites important in malignant transformation. (60 references)

0614 THE ROLE OF CYCLIC AMP IN MALIGNANT TRANSFORMATION. (Eng.) Pastan, I. (Natl. Cancer Inst., Bethesda, Md.). *Am. J. Clin. Pathol.* 63 (5):669-670; 1975.

Evidence indicating that many properties of transformed cells are due to low levels of cyclic adenosine monophosphate (cAMP) is briefly summarized. When treated with cAMP, cAMP analogs, or agents that raise intracellular cAMP levels, it is observed that many types of transformed cells undergo changes in behavior. Such changes include slower growth, altered appearance, increased adhesiveness to substrate, slower movement, and decreased agglutinability. Measuring the levels of cAMP in numerous normal and transformed fibroblasts reveals that the level of cAMP is directly related to the rate of growth. Transformed cells fail to increase their cAMP levels even when crowded together; this inability to increase cAMP levels is believed to be a key regulatory defect. Membrane-bound adenylate cyclase is frequently altered; increased Km or altered Vmax values are observed. Viruses or carcinogens may act to lower cAMP levels. These levels may also be altered *via* enhanced phosphodiesterase activity. Whereas most studies utilize cultured mouse, rat, and chicken fibroblasts, it is suggested that cAMP effects may be widespread. (8 references)

0615 EPIDEMIOLOGY OF HODGKIN'S DISEASE: REVIEW AND ETIOLOGIC LEADS. (Eng.) Vianna, N. J. (New York State Health Dep., Albany); Davies, J. N. P. *CRC Crit. Rev. Clin. Lab. Sci.* 5(3):245-288; 1975.

Epidemiologic information suggesting a heterogeneity within Hodgkin's disease and evidence of its possible infectious nature is reviewed. Varieties of Hodgkin's disease are condensed into four subtypes: lymphocyte predominance, mixed cellularity, lymphocyte depletion, and nodular sclerosis. However, the major function of the classification is as a prognostic indicator, with an unknown relationship to etiology. Marked variations in incidence patterns of the disease are observed internationally and regionally, suggesting the importance of environmental factors. As distinguished from other lymphomas, Hodgkin's disease displays a characteristic bimodal age-specific incidence curve for each sex. Three different patterns are noted, relating each to the degree of urbanization; this epidemiologic pattern is dynamic, not fixed. Furthermore, there is a reciprocal relationship between Hodgkin's disease in the young adult and childhood groups. Sex ratios vary according to country and age group, but there is a universal predominance in males. Available data on ethnic differences suggests a heterogeneity in Hodgkin's disease, as do analyses of age and sex. Occupational-social factors imply a possible infectious nature. Although studies on the seasonality and time-space clustering of the disease remain inconclusive, a study on Hodgkin's disease incidence as related to schools suggests that horizontal transmission may be an important factor in the pathogenesis of the disease; possibilities of several intermediaries, and the importance of host factors are considered. An hypothesis is presented suggesting the risk of developing the disorder is greater for individuals who previously underwent tonsillectomies than those who did not, which would be compatible with infectious epidemiology. Results of studies suggest that prior tonsillectomies increased the risk by a factor of 2.9. A 3-fold excess of Hodgkin's disease cases among families of Hodgkin's disease is also interpreted as consistent with an infectious etiology, as do studies on time interval and onset of the disease. However, it is suggested that other factors in addition to apparent transmission might be necessary for the disease to occur. There is the additional hypothesis that some association exists between an EBV-like virus and the disease; such an agent could act primarily as a cofactor temporarily depressing cellular immunity and allowing for a role of genetic and environmental factors as well. (138 references)

0616 AN EPIDEMIOLOGICAL EVALUATION OF THE CAUSES OF CANCER OF THE PANCREAS. (Eng.) Wynder, E. L. (Naylor Dana Inst. for Disease Prevention, American Health Foundation, New York, N.Y. 10019). *Cancer Res.* 35(8):2228-2233; 1975.

Recent epidemiologic evidence suggesting a relationship between diet and cigarette smoking to cancer of the pancreas is reviewed. Several prospective and retrospective studies have shown that the risk for pancreatic cancer is increased in cigarette smokers, but not in cigar or pipe smokers. Findings suggesting that dietary factors affect the development of pancreatic cancer include the increase of the cancer among Japanese immigrants to the U.S., the relatively high incidence among American Negroes and Jews, and a positive association between pancreatic cancer and high fat and/or cholesterol diets. Other factors of etiologic interest are the positive correlation between diabetes and cholecystectomy and pancreatic cancer in women, and the greater incidence of the disease (2/1 sex ratio) in men under 50. Two ways in which tobacco carcinogens could reach the pancreas are reflux from the bile duct into the pancreatic duct and distribution through the blood stream. Among the carcinogens that can be distributed by the blood stream are organ-specific nitrosamines, which produce pancreatic lesions in experimental animals. A third mechanism that would account for the increased risk of pancreatic cancer in smokers may be related to elevated serum cholesterol or lipid levels in smokers. The *in vivo* conversion of cholesterol to a cholesterol epoxide may be of etiological significance, although the carcinogenicity of such epoxides for epithelial tissue remains to be established. Two bile acids, lithocholic acid and taurodeoxycholic acid, were recently shown to act as tumor promoters in the colon of rats previously initiated with methylnitrosoguanidine. An attempt should be made in future epidemiological studies to identify reasons for the current

sex ratio and the higher incidence of pancreatic cancer in blacks; studies should also be carried out to verify the data linking diabetes and gallbladder diseases to pancreatic cancer. It is recommended that tobacco manufacturers work toward a further reduction in tar and nicotine levels of cigarettes; food and agriculture industries are urged to work toward a reduction in the cholesterol content of eggs, and a reduction of the fat content of beef and milk. (16 references)

0617 STRUCTURES AND ORGANIZATION OF CELL SURFACE GLYCOLIPIDS DEPENCENCY ON CELL GROWTH AND MALIGNANT TRANSFORMATION. (Eng.) Hakomori, S. I. (Sch. Public Health, Univ. Washington, Seattle). *Biochim. Biophys. Acta* 417(1):55-89; 1975.

A discussion of interrelationships between chemical, enzymatic, and organizational changes of glycolipids, and a correlation of those changes as a function of tumor cell characteristics is presented. Considerations of the chemical change of glycolipid composition in transformed cells include the universal changes observed, simplification of glycolipid patterns due to blocked synthesis, various other changes due to transformation, the change in blood group glycolipids and fucolipids associated with malignant transformation, and chemical patterns and synthesis in tumor cells. The enzymatic basis of the chemical change of glycolipids is investigated using glycosyltransferases and glycosylhydrolases. Studies reveal contact-dependent enhancement of glycolipid synthesis; this is assumed due to the presence of cell surface glycosyltransferases. Investigations on the organization of glycolipids, glycoprotein, and other membrane components reveal the relationship of galactoprotein *a* to growth control and cell contact, the qualitative differences of the lactin-binding protein in normal and transformed cells, the greater exposure of glycolipids in transformed cells, the presence of a specific sialyl-galactosyl or sialyl-*N*-acetyl-galactosaminyl protein in transformed cell surfaces, and the presence of specific surface labels for specific glycolipids. Three general categories of changes are observed: (1) changes in glycolipid composition and synthesis, (2) absence of cell contact-dependent enhancement of glycolipid synthesis, and (3) deletion of a high molecular weight galactoprotein *a* and exhibition of a higher extent of glycolipid exposure on transformed cells. (121 references)

0618 CHALONE CONTROL MECHANISMS. (Eng.) Bullough, W. S. (Mitosis Res. Lab., Birkbeck Coll., Univ. London, England). *Life Sci.* 16(3):323-330; 1975.

The mechanism of tissue production and growth control by tissue-specific mitotic inhibitors, the chalones, is reviewed. In the best studied chalone mechanism, that of the epidermis, the basal mitotic rate is inversely proportional to the chalone effectiveness. A cell becomes post-mitotic when its internal chalone effectiveness becomes supercritical; the speed of its subsequent aging then depends on the distal chalone effectiveness, being again inversely proportional. Hence, the chalone inhibits both basal cell production and distal cell loss. Under normal conditions, a changed mitotic rate due to changed chalone effectiveness should not change the epidermal thickness. The system is stable while distal chalone concentration remains above the critical level, assuring post-mitosis; below that level, distal cells start mitosis, proliferate, the balance breaks, and cancer results. Such chalone systems seem to be universal, although chalone effectiveness may be so high on some tissues that cell production and aging are equally inhibited, and no cells are lost. The manner of failure of the chalone mechanism in cancer indicates that tumor cells contain abnormally low concentrations of the chalone of their tissue of origin, due not to a failure of chalone synthesis but to excessive chalone loss across abnormal cell membrane. Abnormal leakage of chalone into body space results in increased blood chalone concentration, increased mitotic inhibition in the tissue of origin and the tumor, and consequently to the tumor sigmoidal growth rate. Tissue specificity of chalone production and action remains in anaplastic tumors. Whereas theory predicts chalone treatment should merely cause tumor mass to plateau, the actual findings indicate tumor growth is related to increased potential for cell production and to reduced potential for cell survival. Hence, mitotic advantage reduced by chalone treatment may ensure the destruction of the tumor by adverse factors. (28 references)

0619 SERUM AND URINE POLYAMINES IN CANCER. (Eng.) Savory, J. (Div. Clin. Chem., Univ. North Carolina, Chapel Hill); Shipe, J. R. *Ann. Clin. Lab. Sci.* 5(2):110-114; 1975.

Elevated concentrations of urinary polyamines are found associated with various types of solid tumors and leukemias; these include rectal carcinoma, lymphosarcoma, Hodgkin's disease, osteogenic sarcoma, and acute myelocytic leukemia. Accumulation of polyamines, implicated in the regulation of RNA synthesis, is found in both normal and neoplastic rapidly growing tissues. An initial colorimetric method for the quantitation of serum spermine suffered a lack of specificity. Greater specificity, yet relative insensitivity, was obtained through ion-exchange chromatography. More recent techniques include paper electrophoresis, paper chromatography, thin-layer chromatography, serological methods, and enzyme degradation; however, sensitivity and specificity remain insufficient. Gas-liquid chromatography and automated ion-exchange techniques offer greatly improved accuracy and sensitivity. An amino acid analyzer is utilized to improve the speed and sensitivity of the cation-exchange method. Gas chromatography-mass spectrometry and the use of ion detection or mass fragmentography, combined with stable isotopes of the amines as internal standards also offer sensitive and specific means of detection. Normal values for the measurement of serum and urinary polyamines are quoted. Whereas the level of urinary spermine is overestimated by the high voltage electrophoresis method, there is generally good agreement between the various analytical ap-

proaches to estimating polyamine levels. In urine excretion from patients with diagnosed leukemia, lymphoma and metastatic solid tumors, elevation in spermidine and spermine are noted most frequently, with levels being several-fold the normal range. Although there are demonstrated elevations of polyamines of cancer patients, no trends are observed. (31 references)

0620 CANCER RISK REDUCED. (Eng.) Anonymous. *Aust. Mach. Prod. Eng.* 28(5):21; 1975. (No references).

0621 ASBESTOS AS AN AIR POLLUTANT: ITS ROLE IN HUMAN PATHOLOGY. (Fre.) Fréour, P. (Laboratoire d'Hygiène de la Faculté de Médecine, Place de la Victoire, 33000 Bordeaux, France); Pierron, J.-R. *Bull. Acad. Nat. Med., Paris* 159(3):171-175; 1975. (20 references)

0622 ASBESTOSIS -- A PERSONAL VIEW. (Eng.) Smither, W. J. (Cape Asbestos Co. Ltd., London, England). *Community Health* 6(5):267-272; 1975. (4 references)

0623 BURKITT'S LYMPHOMA IN A PATIENT TREATED FOR HODGKIN'S DISEASE: ONE OR TWO DISEASES [letter to editor]. (Eng.) Sartiano, G. P. (Sch. Med., Univ. Pittsburgh, Pa.); Magrath, I. T. *N. Engl. J. Med.* 292(25):1353; 1975. (2 references)

0624 THE MARKET BASKET: FOOD FOR THOUGHT. (Eng.) Deichman, W. B. (Univ. Miami Sch. Med., Fla.). *Am. Ind. Hyg. Assoc. J.* 36(6):411-429; 1975. (56 references)

0625 PRENATAL OESTROGEN -- CONTINUED. (Eng.) Anonymous. *Lancet* (7913):960-961; 1975. (10 references)

0626 MEDICAL PROBLEMS FROM MODERN DIET. (Eng.) Yudkin, J. (Dep. Nutr., Univ. London, England). *J. R. Coll. Physicians Lond.* 9(2):161-164; 1975. (12 references)

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0655 STUDIES ON LARGE DNA PLASMIDS OF *AGROBACTERIUM TUMEFACIENS* [abstract]. (Eng.) Hernalsteens, J. P. (Laboratorium voor Genetische Virologie, Vrije Universiteit Brussel, Belgium); Engler, G.; Van Larebeke, N.; Van Montagu, M.; Schell, J. *Arch. Int. Physiol. Biochim.* 83(2):368-369; 1975. (5 references)

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0657 *IN VIVO* TESTING OF HYPOXIC RADIOSENSITIZERS USING THE KHT MURINE TUMOUR ASSAYED BY THE LUNG-COLONY TECHNIQUE. (Eng.) Rauth, A. M. (Ontario Cancer Inst., Toronto, Canada); Kaufman, K. *Br. J. Radiol.* 48(567):209-220; 1975.

The KHT transplantable tumor of C3H mice was used as a model tumor for the *in vivo* study of 11 hypoxic cell radio sensitizers comprising four nitrofuran, five nitrobenzene, and two nitroimidazole derivatives, which had been shown to be effective on hypoxic mammalian cells *in vitro*. The study population comprised four groups of mice: 1) control, phosphate-buffered saline, only; 2) sensitizer (i.p.) but no radiation; 3) phosphate-buffered saline and a test dose of 2000 or 2500 rads of ^{137}Cs γ rays at 96 rads/min whole-body, zero or 15 min after injection; and 4) sensitizer plus the test dose of radiation. After irradiation, cell viability was determined using the *in vivo* lung-colony assay. Two of the sensitizers 2-methyl-5-nitroimidazole-1-ethanol and ethyl [2-(2'-methyl-5'-nitro-1'-imidazolyl) ethyl] sulfone, showed signs of hypoxic cell sensitization *in vivo* when given i.p. 1500 and 750 mg/kg, resp. Failure to obtain sensitization with some of the other compounds may be due to the low solubility of the drugs in aqueous medium, which made it impossible to give high drug levels, or to the failure of the compoundto reach or penetrate the tumor. When the same compounds were given by intra-tumor injection, none of the compounds that failed to work by i.p. injection worked under these conditions. However, preliminary testing of p-nitro-3-dimethyl-propriophenone hydrochloride indicated that when it was injected directly into the tumor and irradiation was completed within ten min after injection, appreciable sensitization was obtained. Both 2-methyl-5-nitroimidazole-1-ethanol and ethyl [2-(2'-methyl-5'-nitro-1'-imidazolyl)ethyl] sulfone at 1500 mg/kg and 750 mg/kg, resp. gave an enhancement ratio of 1.5 for a chronically hypoxic cell population in this tumor. Measurements of plasma levels of 2-methyl-5-nitroimidazole-1-ethanol and enhancement ratios obtained in this *in vivo* system seem to be in agreement with published *in vitro* and *in vivo* results.

0658 A NEW COMMON MARKER FOR PREMALIGNANT AND MALIGNANT HEPATOCYTES INDUCED IN THE RAT BY CHEMICAL CARCINOGENS. (Eng.) Okita, K. (Temple Univ. Sch. Med., Philadelphia, Pa.); Kligman, L. H.; Farber, E. *J. Natl. Cancer Inst.* 54(1):199-202; 1975.

An apparently new common antigen tentatively called preneoplastic has been identified. Intensive positive fluorescent staining was observed in all early or late hyperplastic nodules and in every primary hepatoma induced by N-2-fluorenylacetamide, ethionine, 3'-methyl-4-dimethylaminoazobenzene, dimethylnitrosamine, or diethylnitrosamine in three strains of rats (CFN, F344, and BUF). Normal rat livers, fetal livers, liver surrounding nodules or cancer, amniotic fluid, or cholangioma did not show any specific fluorescence. In the stained cells, specific fluorescence was observed regularly as fine granules in the cytoplasm. Nuclei were uniformly negative. Virtually every hepatocyte in the hyperplastic nodules and in primary hepatomas showed staining. The antigen was not detected in the sera of rats bearing preneoplastic antigen-positive nodules or hepatomas. It also could not be found at the current level of detection in livers of rats treated acutely with carbon tetrachloride, dimethylnitrosamine, or α-naphthylisothiocyanate, in regenerating liver of rats at three days, or in three types of neoplasms in rats other than liver cancer. The two most probable hypotheses under serious study are that the preneoplastic antigen is 1) a viral antigen, either from an RNA or a DNA virus, or 2) an antigen that appears in response to the interruption of hepatocyte differentiation seen in the nodule populations. It is suggested that the observations with preneoplastic antigen, regardless of its fundamental nature, offer the strongest evidence of a unifying link between the hyperplastic hepatocyte populations presumed to be premalignant and the malignant neoplastic hepatocytes in liver cancer. This antigen also offers a new marker that could prove useful as a quantitative index of early changes related to cancer development.

0659 CARCINOGENESIS *IN VITRO*. II. CHEMICAL TRANSFORMATION OF DIPLOID HUMAN CELL CULTURES: A RARE EVENT. (Eng.) Igel, H. J. (The Children's Hosp., Buchtel Ave. and Bowery St., Akron, Ohio 44308); Freeman, A. E.; Spiewak, J. E.; Kleinfeld, K. L. *In Vitro* 11(3):117-129; 1975.

Seventy-five human cell strains were subjected to a number of chemical carcinogens, including urethane and polycyclic hydrocarbons, in an attempt to produce an *in vitro* chemically transformed human cell line. Fresh tissue specimens were grown in Eagle's minimum essential medium. Chemical carcinogens were fed to primary cultures, if possible, or at an early subdivision; they were applied on the third and fifth day after plating or division. On the eighth day, the carcinogens were washed out, and the cultures were refed with growth medium. Treatment with urethane (1.1×10^{-2} M) was repeated for three subdivisions, and treatment with 3-methylcholanthrene (3.7×10^{-5} to 3.7×10^{-7} M) was repeated for six subdivisions. Other chemical treatments also were repeated for 3-6 subdivisions, and included the following: benzo(α)pyrene, 4×10^{-5} to 4×10^{-6} M; benzanthracene, 4.4×10^{-5} M; diethylnitrosamine, 1×10^{-3} M; 4-nitroquinoline-1-oxide, 7×10^{-6} to 8×10^{-7} M; β-propiolacetone, 2.5×10^{-4} M; and 7,12-dimethylbenzanthracene, 3.9×10^{-5} to 3.9×10^{-7} M. The normal characteristics of the cell cultures were altered by the chemical agents in only two cases; microscopic foci of morphologically altered cells appeared in two cell lines derived from neurofibromas of siblings with von Recklinghausen's disease. Attempts to isolate continuous cell lines from these altered foci were successful in only two cases. These continuous cell lines demonstrated altered morphology, loss of contact inhibition, accelerated growth rate, and attained over 240 generations in a period of 140 wk. Untreated control cultures became terminal by the 20th generation. Giemsa banding procedures showed that the chromosomal complement consisted of heteroploid

human chromosomes. When the transformed cells were inoculated sc (10^7 cells/0.5 ml) into ten newborn NIH Swiss mice, six developed tumor nodules at the site of inoculation within one week. No nodules could be produced with nontransformed cells from the same patients. Two cell lines derived from the tumor nodules were morphologically identical to the parent transformed cells and had the same karyotypic characteristics as the parent human cell culture used for inoculation. It is concluded that chemical transformation of human cells is difficult to induce; however, selection of genetically predisposed cells and prolonged, intermittent, and repeated chemical treatment may be important factors in achieving transformation.

0660 ALKALINE PHOSPHATASE ACTIVITY IN HYPERPLASTIC AND NEOPLASTIC URINARY BLADDER EPITHELIUM OF MICE FED 2-ACETYLAMINOFLUORENE. (Eng.) Highman, B. (Univ. Arkansas Med. Cent., Little Rock); Frith, C. H.; Littlefield, N. A. *J. Natl. Cancer Inst.* 54(1):257-261; 1975.

The effect of p.o. 2-acetylaminofluorene (2-AAF) on alkaline phosphatase activity in the urinary bladder was studied in BALB/c mice. Male and female mice were maintained for seven or 19 months on diets containing 100, 250, or 500 ppm 2-AAF. The bladder epithelia were stained for alkaline phosphatase using a modification of the cobalt sulfide method. Bladder epithelial hyperplasia was observed in 22/24 mice given 2-AAF for seven months, and in 20/21 animals given 2-AAF for 19 months. Transitional cell carcinomas were also observed in 12 of the mice given 2-AAF for 19 months; no such tumors were noted in the females treated with the lowest dose for this period. Marked alkaline phosphatase activity was demonstrated in the bladder epithelia of the untreated controls, this activity being slightly to moderately reduced in the lower epithelial layers of the mice treated with 2-AAF for seven months. The activity of this enzyme was markedly reduced and confined largely to the upper layers in the mice treated with 2-AAF for 19 months. There was no activity in the transitional cell carcinomas except for some focal activity in a few tumor masses in 11/12 mice bearing tumors. Alkaline phosphatase activity was usually slightly to moderately increased in the epithelial stroma after seven months of treatment, and was markedly increased after 19 months. The results suggest that aging and neoplasms may contribute to this increase in stromal alkaline phosphatase activity. They also indicate that loss of alkaline phosphatase activity in the bladder epithelium may be a frequent precursor of transitional cell carcinomas in BALB/c mice and perhaps in man.

0661 CHANGES OF TISSUE WATER PROTON RELAXATION RATES DURING EARLY PHASES OF CHEMICAL CARCINOGENESIS. (Eng.) Floyd, R. A. (Johnson Res. Found., Univ. Pennsylvania, Philadelphia); Yoshida, T.; Leigh, J. S., Jr. *Proc. Natl. Acad. Sci. USA* 72(1):56-58; 1975.

Water proton spin lattice relaxation rate (T_1) was determined on the liver, spleen, and blood serum of male Wistar rats experiencing early phases of chemical carcinogenesis. The rats were fed a fast acting carcinogen, 3'-methyldimethylaminoazobenzene, and a slower acting carcinogen, 2-acetylaminofluoren freely at a level of 0.06% (w/w) in a basal syntheti diet for up to four wk. T_1 values were determined in about 0.3 g samples of liver and spleen and in about 50 μliter of serum by a previously described method. T_1 of blood serum and liver tissue (0.81 and 0.195, resp.) was significantly higher after fou wk of 3'-methyldimethylaminoazobenzene feeding than that of the controls (0.70 and 0.165, resp.). This was not the case with 2-acetylaminofluorene (0.78 an 0.166 for blood serum and liver, resp. compared to 0.77 and 0.172, resp., for the controls). The blood serum T_1 increase reflected the onset of liver nodulation. Liver T_1 values increased as the degree of nodulation increased. Blood serum T_1 correlated inversely with protein content and directly with water content. Liver T_1 values correlated with wate content, but this was not true for spleen T_1 values. Spleen T_1 values were significantly lower than controls at the earliest sampling date for each carcinogen: one wk for 3'methyldimethylaminoazobenzene (0.240 and 0.273, resp.) and four wk for 2-acetylaminofluorene (0.214 and 0.273, resp.). The spleen T_1 decrease paralleled an increase of iron detectable by electron spin resonance in this tissue. It is suggested that spleen T_1 decreases are probabl not unique to chemical carcinogenesis. It is conclu ed that there is an increase in blood serum T_1 long before *bona fide* liver tumors are present. This fact, coupled with the convenience of blood serum monitoring, suggests the potential use of blood T_1 determination as a possible screening technique.

0662 MODIFICATION OF HEPATIC RIBONUCLEIC ACID POLYMERASE ACTIVITIES BY *N*-HYDROXY-2-ACETYLAMINOFLUORENE AND *N*-ACETOXY-2-ACETYLAMINOFLUORENE. (Eng.) Glazer, R. I. (Dep. Pharmacol., Emory Univ., Atlanta, Ga.); Glass, L. E.; Menger, F. M. *Mol. Pharmacol.* 11(1):36-43; 1975.

The template activity of DNA and the activities of crude and partially-purified RNA polymerases from hepatic nuclei isolated from partially-hepatectomize rats treated with *N*-hydroxy-2-acetylaminofluorene (*N*-hydroxy-AAF) were studied, and the relative abilities of rat liver and *Escherichia coli* RNA polymerases to discriminate *N*-acetoxy-AAF-altered DNA template activity were compared. Male Sprague-Dawley rats were partially hepatectomized and injected i.p. 18 hr later with 20 mg/kg *N*-hydroxy-AAF or the propylene glycol vehicle alone (controls); the animals were killed two hr later and the liver RNA polymerase and DNA prepared. In the second experiment, hepatic DNA was incubated *in vitro* at 22 or 37 C with *N*-acetoxy-AAF; this DNA was then dissolved in an assay mixture to measure rat liver and *E. coli* RNA polymerase activity. Treatment of the partially hepatectomized rats with *N*-hydroxy-AAF inhibited the activities of the nucleolar and nucleoplasmic RNA polymerase in the hepatic nuclei. DEAE-Sephadex chromatography of the nuclear RNA polymerases obtain ed from partially hepatectomized rats treated with *N*-hydroxy-AAF revealed stimulation in the activity

of RNA polymerase I (nucleolar) and inhibition and an altered activity profile of RNA polymerase II (nucleoplasmic). No alterations were observed in the template activity of rat liver DNA from similarly treated animals. Assessment of the template activities of hepatic DNA after *in vitro* incubation with *N*-acetoxy-AAF at 22 C indicated that the RNA polymerase II activity was affected to a greater degree than that of RNA polymerase I or *E. coli* RNA polymerase, particularly at lower carcinogen concentrations. In contrast, reaction of hepatic DNA with *N*-acetoxy-AAF at 37 C resulted in a 20- to 30-fold reduction in the concentration of activated carcinogen necessary to effect a 50% reduction in the rat liver or *E. coli* RNA polymerase activities. Whereas inhibition of nucleoplasmic RNA polymerase can account for the impairment in the synthesis of the extranucleolar species of RNA, it appears that inhibition of the synthesis of ribosomal RNA is not a direct result of the reduction in nucleolar RNA polymerase or DNA template activity.

0663 *IN VITRO* METABOLIC CONVERSION OF AFLATOXINS AND BENZO(*a*)PYRENE TO NUCLEIC ACID-BINDING METABOLITES. (Eng.) Gurtoo, H. L. (Roswell Park Mem. Inst., Buffalo, N. Y.) Dave, C. V. *Cancer Res.* 35(2): 382-389; 1975.

The effects of inducers and inhibitors of microsomal mixed-function oxygenase and the relationship of aflatoxin structure to the generation of the reactive metabolites that bind to DNA and RNA were investigated and the enzymatic pathways for the formation of DNA-binding metabolites from aflatoxin B_1 and from benzo(*a*)pyrene were compared. Hepatic microsomes were isolated from untreated, phenobarbital-treated (40 mg/kg i.p.), and methylcholanthrene-treated (15 mg/kg, i.p.) rats. In the standard assay, [^{3}H] aflatoxin B_1 was incubated with rat hepatic microsomes, rat liver RNA, or native calf thymus DNA in the presence or absence of a nicotinamide adenine dinucleotide phosphate-generating system. An aflatoxin B_1 metabolite became covalently bound to rat liver RNA and calf thymus DNA *in vitro* and formed complexes with increased spectral absorbance in the 360 nm region. The formation of such complexes, which was microsome- and reduced nicotinamide adenine dinucleotide phosphate-dependent, was inhibited by β-diethylaminoethyl diphenylpropylacetate-HCl, and by CO and N_2, when the later were used to replace the gas phase of the incubations. The formation of the complexes was enhanced about 2-fold with microsomes from phenobarbital-treated rats but not from 3-methylcholanthrene-treated rats. More binding was observed with DNA than with RNA. In the presence of reduced nicotinamide adenine dinucleotide phosphate and microsomes from phenobarbital-treated rats, aflatoxin G_1 was also converted into metabolite(s) that became covalently bound to nucleic acids and formed complexes with increased spectral absorbancies in the 360 nm region. Under the same conditions, aflatoxin B_2, aflatoxin $B_{2}a$, aflatoxin G_2, and "Compound 11", which lack a C2-C3 double bond, did not show any noticeable binding to either DNA or RNA. These data support the concept that the microsomal mixed-function oxygenase-catalysed oxidation of the C2-C3 double bond of aflatoxins is a prerequisite for the formation of nucleic acid-binding metabolites. In assays involving the formation of DNA-binding metabolites from benzo(*a*)pyrene, microsomes from 3-methylcholanthrene-treated rats were 12- and 5-fold more active than microsomes from untreated and phenobarbital-treated rats, resp. This suggests that different enzymes in hepatic microsomal mixed-function oxygenase complex are involved in the generation of reactive metabolites from various polycyclic hydrocarbons.

0664 HEPATOCELLULAR CARCINOMA: RELATION TO ALCOHOL, HB-ANTIGEN AND ALPHA-FETOPROTEIN. (Eng.) Dourdourekas, D. (Hektoen Inst. Med. Res., Cook Cty. Hosp., Chicago, Ill.); Villa, F.; Szanto, P. B.; Steigmann, F. *Am. J. Gastroenterol.* 63(4): 307-311; 1975.

The relationship of alcoholic liver disease, hepatitis B antigen and alpha-fetoprotein to hepatocellular carcinoma was studied in 16 patients with hepatocellular carcinoma over an 18-month period. The definitive diagnosis of hepatocellular carcinoma was established by liver biopsy: five were obtained blindly by a percutaneous approach with a Menghini needle; the other 11 were obtained during a peritoneoscopic examination. The biopsy of one patient was negative for hepatocellular carcinoma, but during peritoneoscopy the liver was found to be cirrhotic with many areas highly suggestive of carcinoma. Thirteen patients had coexisting cirrhosis of the liver. The sera of seven out of 14 patients were positive for alpha-fetoprotein; three of 12 tested were positive for hepatitis B-antigen. The sera of two patients were positive for both alpha-fetoprotein and hepatitis B-antigen. There was no difference in the survival rate between the patients who had positive or negative alpha-fetoprotein, or between those with or without positive hepatitis B antigen. There was also no difference in the survival rate between those with or without coexistent cirrhosis. The period the patients survived after the diagnosis of hepatoma was established was very short, ranging from 1-14 months, with an average of three months. The finding of definite cirrhosis of various degrees in 13 of 16 patients with primary cancer of the liver suggests a strong relation between these two types of lesions. It is suggested that alcohol be declared a carcinogen.

0665 INTERACTION OF AFLATOXICOSIS WITH HEAT STRESS. (Eng.) Wyatt, R. D. (Dept. Poult. Sci., North Carolina State Univ., Raleigh); Thaxton, P.; Hamilton, P. B. *Poult. Sci.* 54(4): 1065-1070; 1975.

The effects of high temperature stress of cloacal temperature, body fat, and serum glucose in aflatoxin-treated chickens were investigated. Aflatoxicosis was induced in 1-day-old males by incorporating various doses of aflatoxin (0, 0.625, 1.25, 2.5, 5.0 and 10.1 μg/g) in a diet from which all medications had been omitted. Four groups of ten birds were fed each diet for three weeks. The mean

survival times of 3-wk-old chickens fed the aflatoxin-containing diets since hatching were determined at 37 C and 45% relative humidity and at 40 C and 45% humidity. Both serum glucose and total body fat were decreased significantly by doses $\geq$ 2.5 μg/g. Cloacal temperature was decreased slightly but significantly in chickens fed 5 or 10 μg/g for 12 days or longer. The mean survival time of birds exposed to a heat stress of 40 C and 45% relative humidity did not vary with the dose of aflatoxin. A milder stress of 37 C and 45% relative humidity caused chickens fed aflatoxin at doses $\geq$ 2.5 μg/g to show decreased survival times in comparison to the controls. These data might be explained by assuming that the lessened burden of body fat during aflatoxicosis accounts for the increased survival time in a severe heat stress (43 C and 45% relative humidity), but that other parameters related to physiological stress play a dominant role during a less severe but more prolonged heating episode. It is also suggested that the hypoglycemia, hypothermia, and lessened body fat account for the previously reported increased sensitivity to a lethal cold exposure during aflatoxicosis.

0666 EFFECT OF DIETARY FAT UPON AFLATOXICOSIS IN RATS FED TORULA YEAST CONTAINING DIET. (Eng.) Wells, P. (Sch. Public Health, Univ. California, Los Angeles); Aftergood, L.; Parkin, L.; Alfin-Slater, R. B. *J. Am. Oil Chem. Soc.* 52(5): 139-143; 1975.

The interrelationships between Torula yeast, vitamin E, and dietary fat source on aflatoxin toxicity were studied in male weanling rats of the former USC strain. Rats were fed Torula yeast-containing basal diets which included 1.7 ppm aflatoxin B_1 with either lard, corn oil or no fat, and with or without vitamin E supplements for three months. After that, the respective diets without aflatoxin were fed for nine months. At the beginning, all groups included 10 rats; high mortality (50%) among the vitamin E-deficient rats, however, forced their premature sacrifice after 4-10 wk. Although the vitamin E-deficient, aflatoxin-treated rats had lower wt gains than did the vitamin E-deficient controls, they lived twice as long. Regardless of the dietary fat source, the kidneys and adrenals of these rats were significantly heavier than the controls, and plasma cholesterol levels were elevated. Increased amounts of liver lipid were observed in response to aflatoxin in both corn oil-fed and fat-deficient rats. No such differences were observed in the responses of the vitamin E-supplemented groups to aflatoxin. On the corn oil diet, aflatoxin administration resulted in an increased deposition of polyunsaturated fatty acids in cholesteryl ester and phospholipid fractions in livers of vitamin E-deficient rats and the phospholipid fraction of vitamin E-sufficient rats. The vitamin E-deficient rats had necrosis of the liver, which was alleviated when aflatoxin was included in the diet, and calcification of the kidneys, which was potentiated by the dietary aflatoxin. In animals maintained on vitamin E-sufficient diets for one yr, growth was depressed with aflatoxin, with the greatest depression occurring in the group fed corn oil. Spleen wt was decreased in all groups given aflatoxin. When aflatoxin was fed with lard, the cholesterol ester, triglyceride, and free fatty acid fractions of plasma had decreased amounts of the C20:4 acid. In the liver phospholipids, there were increased levels of mono- and polyunsaturated fatty acids and decreases in the saturated fatty acids. All of the animals receiving aflatoxin exhibited severe necrosis and tumor formation in the kidneys; the animals fed lard had the highest level of involvement and those in the fat-free group the least. Liver pathology was the least marked among the rats fed the fat-free diet. The quality of the dietary protein is apparently important in establishing the response of an animal to aflatoxin. Interactions between the fat and protein sources in the diet may alter the animal's susceptibility to dietary contaminants.

0667 INDUCTION OF CHOLANGIOCARCINOMA FOLLOWING TREATMENT OF A RHESUS MONKEY WITH AFLATOXIN. (Eng.) Tilak, T. B. G. (Natl. Inst. Nutr., Hyderabad, India). *Food Cosmet. Toxicol.* 13(2): 247-249; 1975.

The carcinogenic activity of aflatoxin was observed in a monkey treated with the substance over a long period. A female rhesus monkey weighing 2.0 kg was treated with a crystalline preparation of mixed aflatoxins (B_1 44%, G_1 44%, and B_2 and G_2 2%) for 5.5 yr. The preparation was administered i.m. five days/week for the first yr in a dose of 50 μg/day for one month and 100 μg/day thereafter. A dose of 100 μg/day was given p.o. on five days/week for the remaining 54 months. A massive globular greyish-white hard mass occupying the lateral portion of the right lobe of the liver was observed at autopsy and was attached to the diaphragm. The weight of the liver with the tumor was 465 g. There were satellite nodules about 1 cm in diameter. Enlargement of lymph nodes in the porta hepatis was observed. On the basis of the morphology, the tumor was designated an adenoacanthoma of the intrahepatic bile ducts, or possibly an adenosquamous cholangiocarcinoma. The tumor was presumably induced by aflatoxin for the following reasons: (1) aflatoxin is one of the most potent hepatocarcinogens known, (2) in lower animals, cholangiocarcinomas constitute one of the known sequelae of chronic aflatoxicosis, and (3) this type of tumor does not ordinarily arise spontaneously in monkeys. The author suggests that aflatoxin consumption may be related to human primary liver cancer in some areas, (e.g. Swaziland and Thailand), and recommends the review of safe amounts of these toxins in human food.

0668 CELLULAR PHOSPHORYLATION OF 1-β-D-ARABINOFURANOSYLCYTOSINE AND 5-AZACYTIDINE WITH INTACT FIBROSARCOMA AND LEUKEMIC CELLS. (Eng.) Lee, T. (Univ. Southern California Sch. Medicine, Los Angeles, Calif. 90033); Karon, M.; Momparler, R. L. *Cancer Res.* 35(9):2506-2510; 1975.

The kinetics of the phosphorylation of 1-β-D-arabinofuranosylcytosine and 5-azacytidine by $A(T_1)Cl$-3 ham-

ster fibrosarcoma cells and L5178Y murine leukemic cells were investigated using intact cells. Radioactive nucleosides [^{3}H]cytidine, [^{3}H]deoxycytidine, and [^{3}H]uridine were added to cell suspensions of fibrosarcoma (5 x 10^7 cells/5 ml) and leukemia (8 x 10^6 cells/5 ml) and radioactivity was measured after 30 min at 37 C. The rate of cellular phosphorylation of uridine, deoxycytidine, and deoxythymidine at concentrations of 5-100 μM was linear for 60 min. An arabinofuranosylcytosine-resistant clone of A(T_1)-Cl-3 hamster fibrosarcoma cells was obtained by single-cell isolation from a population of wild-type cells maintained in the presence of 200 μM arabinofuranosylcytosine for 14 days. The cellular phosphorylation of both 1-β-D-arabinofuranosylcytosine and 5-azacytidine appeared to follow Michaelis-Menten kinetics. The apparent K_m value for 1-β-D-arabinofuranosylcytosine in the fibrosarcoma and leukemic cells was about 40 μM, whereas the apparent K_M values for 5-azacytidine in these cells were about 1.3 and 0.41 mM, respectively. Deoxycytidine and cytidine were potent competitive inhibitors of the phosphorylation of 1-β-D-arabinofuranosylcytosine and 5-azacytidine, respectively. The 1-β-D-arabinofuranosylcytosine-resistant clone of fibrosarcoma cells exhibited a higher K_m value for both 1-β-arabinofuranosylcytosine and deoxycytidine than the wild-type fibrosarcoma cells. There appears to be a good correlation between the enzymatic and cellular data on the phosphorylation of 1-β-D-arabinofuranosylcytosine, but not on the phosphorylation of 5-azacytidine. This may be related to the chemical instability of 5-azacytidine or to the effect of this analog on other areas of cellular metabolism.

0669 POSSIBLE COCARCINOGENIC EFFECTS OF COFFEE CONSTITUENTS. (Eng.) Challis, B. C. (Dep. Org. Chem., Imperial Coll., London, England); Bartlett, C. D. *Nature* 254(5500):532-533; 1975.

The formation of N-nitrosamine from nitrite salts and secondary amines at gastric pH in the presence of readily oxidized phenols was investigated. In the absence of phenols, the more basic secondary aliphatic amines, such as dimethylamine and piperidine, reacted sluggishly with aqueous HNO_2. The addition of even 1 mM 4-methylcatechol increased the rate of N-nitrosopiperidine formation by a factor of at least 1000. The addition of 10 mM chlorogenic acid, a very substantial (about 13%) soluble constituent of coffee, led to a maximum 56% N-nitrosopiperidine formation in ten min at pH 4 and 25 C (compared with 42% for 10 mM 4-methylcatechol). Both 4-methylcatechol and chlorogenic acid appeared to be very powerful catalysts for N-nitrosamine formation in aqueous media under mildly acidic conditions. The experimental conditions and reactant concentrations were not appreciably dissimilar from those expected for the human stomach following the ingestion of food containing 200 ppm $NaNO_2$ (about 3 mM) and a single cup of coffee (about 7 mM in chlorogenic acid). The implication is that coffee and other foodstuffs, containing readily oxidized phenolic materials, may significantly increase human exposure to carcinogenic nitrosamines by catalyzing their formation in the digestive tract.

0670 COFFEE DRINKING AND CANCER OF THE LOWER URINARY TRACT. (Eng.) Simon, D. (Harvard Sch. Public Health, Boston, Mass.); Yen, S.; Cole, P. *J. Natl. Cancer Inst.* 54(3):587-591; 1975.

Coffee drinking habits of urban white women from Massachusetts and Rhode Island were studied for a possible relationship to lower urinary tract (LUT) cancer. Patients with LUT cancer were chosen from the pathology log book and diagnostic index of participating hospitals. Three white women were chosen as controls for each case. Data on coffee drinking and related habits were obtained by mail questionnaire for 135 women with LUT cancer and 390 controls. The questions included age when coffee drinking and cigarette smoking began; usual daily consumption of coffee, cigarettes, and tea; occupation; coffee additives used; and type and strength of coffee. The risk ratio for women who drank one or more cups of coffee per day was 2.1 when compared to women who drank no coffee or less than one cup per day. However, the amount of coffee consumed showed no relationship to dose-response. The type of coffee, the strength of the coffee or the use of creamers or sugar substitutes (i.e. saccharin or cyclamates) was not associated with increased risk of disease. The risk of LUT cancer for cigarette smokers as compared to non-smokers was 1.6. The results suggest that the association of coffee drinking with LUT cancer is noncausal.

0671 ACTION OF CYTOCHALASIN D ON CELLS OF ESTABLISHED LINES. III. ZEIOSIS AND MOVEMENTS AT THE CELL SURFACE. (Eng.) Godman, G. C. (Dep. Pathol., Columbia Univ., New York, N.Y.); Miranda, A. F.; Deitch, A. D.; Tanenbaum, S. W. *J. Cell Biol.* 64(3):644-667; 1975.

HeLa, HeLa S3, HEp2, KB, PR105, NCTC Clone 929, XC (from a Rous sarcoma virus-induced rat tumor), MDBK and Vero cell lines grown in monolayer culture in the presence of 0.2 μg/ml cytochalasin D (CD) were studied by electron microscopy; and the induced projection of knobby protuberances at the cell surface (zeiosis) was investigated. HeLa monolayers were pretreated with 10^{-2} M 2-deoxyglucose (DOG), 5 x 10^{-3} M 2,4-dinitrophenol (DNP), 10^{-4} M antimycin A (AMA), 2 x 10^{-5} M carbonyl cyanide m-chlorophenyl hydrazone (CCCP), 2 x 10^{-3} M KCN, 10^{-3} M iodacetamide (IAA), or 10^{-2} M NaN_3 to evaluate the inhibition of zeiosis. Two to three min after application of CD, contraction began, microvilli were withdrawn from the cell surface and umbonate processes developed. These migrated centripetally and coalesced into a single aggregate at the apex of the retracted cell. The protrusions contained chiefly mono- or sub-ribosomes and occasionally other cytoplasmic organelles. Zeiosis was inhibited by metabolic inhibitors when compared to controls (controls, 70% zeiotic cells; DOG, 2%; DNP, 2%, AMA, 4%, CCCP, 3%; KCN, 39%; IAA, 48%; NaN_3, 54%). Epithelial lines were more responsive to CD (HeLa, 0.05 μg/ml to elicit 50% zeiosis; HEp2, 0.05; KB, 0.04) than fibroblastic lines (PR105, 0.33; XC, 0.25) or cells of kidney origin (Vero, 0.35). Zeiosis results from herniation of endoplasm through

the cortex during cell contractions induced by CD, which are inhibited by metabolic inhibitors.

0672 POSSIBLE ROLE OF LYSOSOMAL ENZYMES IN SOME PHARMACOLOGICAL EFFECTS PRODUCED BY BERYLLIUM. (Eng.) Vacher, J. (Centre Recherches Roussel-Uclaf, 93 Romainville, France); Deraedt, R.; Flahaut, M. *Toxicol. Appl. Pharmacol.* 33(2):205-213; 1975.

The effect of beryllium phosphate (BePh) on the mouse liver was examined by determining plasma β-glucuronidase and serum transaminase activities. The i.v. injection of sublethal doses of BePh in Swiss SPF mice produced a biphasic variation of β-glucuronidase activity with maxima at seven and 96 hr. The second peak at 96 hr was accompanied by a large increase in transaminase activity. The injection of doses below 100 to 200 μg Be/kg produced the first phase only, with no change in transaminases 96 hr later. This dissociation illustrates the specific lysosomal effect of Be. The changes in β-glucuronidase activity are thought to be related to the postphagocytic intralysosomal storage of BePh. The first phase is attributed to selective exocytosis of lysosomal enzymes, while the second is attributed to toxic cell damage. The results are considered along with previous work on Be-induced α-macrofetoprotein production and anti-inflammatory effects. These effects could be mediated by synthesis of α-macrofetoprotein and anti-inflammatory substances by the parenchymal cells triggered by the lysosomal enzymes released from the Kupffer cells. It is concluded that i.v. injection of BePh is followed by an increase in plasma β-glucuronidase activity.

0673 STUDIES ON THE MODIFYING EFFECT OF DIMETHYL SULFOXIDE AND OTHER CHEMICALS ON EXPERIMENTAL SKIN TUMOR INDUCTION. (Eng.) Stenback, F. (Univ. Nebraska Med. Cent., Omaha); Garcia, H. *Ann. N.Y. Acad. Sci.* 243:209-227; 1975.

The effects of dimethyl sulfoxide (DMSO) and other chemicals on the induction of skin tumors by treatment with 7,12-dimethylbenz[a]anthracene (DMBA) and 3,4-benzo[a]pyrene (B[a]P) were studied in female Swiss mice. DMBA induced a smaller number of tumors when dissolved in methanol than when dissolved in acetone; acetone or methanol alone failed to induce tumors. B[a]P dissolved in benzene produced about twice as many tumors as it did when dissolved in DMSO; the first tumor appeared at ten weeks in the benzene group as opposed to 20 weeks in the DMSO group. DMSO alone did not cause tumor induction. Irradiation with xenon light significantly decreased the number of tumors induced by DMBA alone, the decrease affecting primarily the number of induced papillomas. Dinitrophenol or DMSO treatment before, during and after initiation did not significantly affect the number of DMBA-induced tumors. Similarly, chlorpromazine application to the skin in addition to DMBA and croton oil did not affect the number of tumors, and no morphological alterations attributable to chlorpromazine application were detected. The results are discussed in terms of the effect of solvents on tumor induction by carcinogenic agents.

0674 STIMULATION BY DIMETHYL SULFOXIDE OF ERYTHROID DIFFERENTIATION AND HEMOGLOBIN SYNTHESIS IN MURINE VIRUS-INDUCED LEUKEMIC CELLS. (Eng.) Friend, C. (Mt. Sinai Sch. Med., City Univ. New York, New York); Scher, W. *Ann. N.Y. Acad. Sci.* 243:155-163; 1975.

The effects of dimethyl sulfoxide (DMSO) on cultured Friend murine leukemia cells and on Swiss mice carrying this leukemia are summarized. Addition of 2% DMSO to the culture medium decreased the nuclear: cytoplasmic ratio, increased the percentage of benzidine-positive cells, increased the number of budding viruses, and decreased the malignancy of the cells. The rates of incorporation of labeled precursors into DNA, RNA and protein were reduced; there was an alteration of polysome patterns; and there were increases in the synthesis of heme, globin, hemoglobin, and δ-aminolevulinate synthetase. Chemotherapy with DMSO (7% in drinking water for 7 day periods alternating with two to three day rest periods) was ineffective in terms of amelioration of disease and prolongation of life. Further, virus isolated from the spleens of treated mice were more numerous and virulent after four weeks of treatment.

0675 THE SPECIFICITY OF DIFFERENT CLASSES OF ETHYLATING AGENTS TOWARD VARIOUS SITES OF HeLa CELL DNA *IN VITRO* AND *IN VIVO*. (Eng.) Sun, L. (Dep. Mol. Biol. Virus Lab., Univ. Calif., Berkeley); Singer, B. *Biochemistry* 14(8):1795-1802; 1975.

The *in vivo* and *in vitro* ethylation of HeLa cell DNA by diethylsulfate, ethyl methanesulfonate, and ethylnitrosourea was studied. HeLa cells were grown in Dulbecco's medium; DNA was extracted from the nuclei; and was dissolved in a buffer containing 20 μl [^{14}C]-diethyl sulfate, [^{14}C]ethyl methanesulfonate, 20 μg [^{14}C]ethylnitrosourea, or 1.2 mg [^{3}H]methylnitrosourea. Ethylated DNA was degraded *via* various combinations of hydrolysis, including neutral or mild acid heating, and enzyme digestion followed by hydrolysis or by alkaline phosphomonoesterase digestion. Separation and identification of the ethyl derivities was accomplished by one- or two-dimensional descending paper chromatography and paper electrophoresis. Significant spontaneous release of ethylated purines was noted. The extent of *in vitro* ethylation of HeLa cell DNA showed a consistent order of reactivity: diethyl sulfate < ethyl methanesulfonate < ethylnitrosourea The extent of nitrogen and oxygen ethylation varied according to the types of ethylating agents used; 83-87% of total ethylation was on the purine bases when diethyl sulfate and ethyl methanesulfonate were used. The extent of *in vivo* ethylation of HeLa cell DNA was much less than *in vitro*, ranging from 0.03-0.14 mM C_2H_5/100 mM P, *versus* 1-2 C_2H_5/100 mM P, with a similar general pattern. Incorporation of the radioactive alkylating agents by normal biosynthetic pathways was also investigated. In general, diethylsulfate and ethyl methanesulfonate ethylated the DNA bases in the following order: 7-ethylguanine > 3-ethyladenine > 1-ethyladenine, 7-ethyladenine > 3-ethylguanine > 3-ethylcytosine, O^6-ethylguanine. In contrast, ethylnitrosourea had a particular affinity for the O^6 position of guanine. A relationship of the formation of DNA

phosphotriesters and relative carcinogenicity is suggested.

0676 INHIBITION OF TUMORIGENESIS BY TOPICAL APPLICATION OF LOW DOSES OF VITAMIN A ACID AND FLUOROURACIL. (Eng.) Prutkin, L. (New York Univ. Med. Cent., N. Y.). *Experientia* 31(4): 494; 1975.

The effects of vitamin A acid by itself or in combination with fluorouracil on tumorigenesis was investigated in 35 male albino rabbits. The inner surface of the right ear auricle of the rabbits was painted twice weekly for three wk with 1% 7,12-dimethylbenzanthracene (DMBA) in lanolin. The rabbits were then divided into seven groups, five animals per group, and subjected to various drug combinations, dosages, and schedules. Biopsies were taken from the treated and untreated rabbit ears and examined by electron microscopy. Rabbits treated with DMBA for three wk had prominent follicular ostea. If no further treatment was given, they had 3-4 tumors per ear three wk later. Those continuing to receive DMBA for an additional three wk had 6-7 tumors per ear. When the sites on the ears were treated for three wk with DMBA and then treated with vitamin A acid (0.1%) and fluorouracil (0.2%) the tumor yield was decreased to 2-3 tumors per ear. Vitamin A acid applications with continued applications of DMBA resulted in 3-4 tumors per ear by the sixth wk. Fluorouracil applications with DMBA had no effect on the number of tumors. When DMBA was applied together with vitamin A and fluorouracil, there were 2-3 tumors per ear. The most striking microscopic observation when vitamin A acid and fluorouracil were applied to the rabbit ear was a marked reduction of heterochromatin in the nuclei. The author suggests that vitamin A and fluorouracil may work synergistically to uncoil the coiled chronema, which results in tumor inhibition, or that vitamin A and/or fluorouracil may complex with DMBA in the vehicle and inhibit the penetration or activity of the carcinogen.

0677 REPARABLE LETHAL DNA DAMAGE PRODUCED BY ENZYME-ACTIVATED 4-HYDROXYAMINOQUINOLINE 1-OXIDE. (Eng.) Tanooka, H. (Natl. Cancer Cent. Res. Inst., Tokyo, Japan); Tada, M.; Tada, M. *Chem. Biol. Interact.* 10(1):11-18; 1975.

The *Bacillus subtilis* transformation system was used to measure the biological activity of DNA and its reparability to determine whether or not DNA inactivated by enzyme-activated 4-hydroxyaminoquinoline 1-oxide (4HAQO) is host reparable. (^{3}H)thymidine-labeled *B. subtilis* DNA was incubated with activated 4HAQO, after which the transforming activity of the DNA (met_{14} marker) was assayed with two different hosts: parental strain *B. subtilis* (thy^- met^-_{14} hcr^+), and a UV-sensitive and host cell reactivation-deficient mutant (thy^- trp^-_2 met^-_{14} hcr^-). Inactivation of the transforming activity proceeded in parallel with the extent of binding of the enzyme-activated 4HAQO to the DNA. The inactivated DNA was susceptible to host-cell reactivation (Hcr) as estimated based on the difference in the survival curves obtained with the Hcr^+ and Hcr^- hosts; this indicated the reparability of the DNA damage. The enzymatic binding of 4HAQO did not induce strand breaks in the DNA, as measured by its sedimentation rate in an alkaline sucrose density gradient. The inactivation efficiency of the activated-4HAQO binding was estimated to be almost the same as that of UV-induced pyrimidine dimers. The results indicate that the activating enzyme is responsible for the intracellular reaction of 4HAQO with DNA.

0678 PANCREATIC NECROSIS AND REGENERATION INDUCED BY 4-HYDROXYAMINOQUINOLINE-1-OXIDE IN THE GUINEA PIG. (Eng.) Reddy, J. K. (Univ. Kansas Med. Cent., Kansas City); Rao, M. S.; Svoboda, D. J.; Prasad, J. D. *Lab. Invest.* 32(1):98-104; 1975.

A model of pancreatic regeneration, dependent on a single i.v. injection of 4-hydroxyaminoquinoline-1-oxide (4-HAQO) was studied. Inbread weanling guinea pigs (NIH strain 13) were injected with a solution of the drug in 0.0005 N HCl at doses of 6.25, 15, 20, 22.5, 25, 50, and 100 mg/kg or HCl only. For autoradiography, two to three animals given the 22.5 mg/kg dose received 1 µCi/g ^{3}H-thymidine one hr before sacrifice at 48, 56, 60, 66, 72, 84, and 96 hr after injection. Two to eight guinea pigs at each dosage were killed. Pancreas and liver were fixed and stained. Amylase concentrations of pancreatic homogenate and serum were determined at various intervals after the 22.5 mg/kg dose. Animals given the 100 mg/kg dose died within 24 hr; those given 50 mg/kg developed severe pancreatic damage 30-44 hr after injection, and died. Animals given the 25-20 mg/kg doses showed pancreatic edema at 18 hr and showed pinhead-sized foci of fat necrosis by 48 hr. Microscopically, there were foci of acinar cell necrosis and zymogen granule reduction within 18 hr. By 36 hr, cells lining the pancreatic acinus became necrotic and by 48 hr the acinar and lobular architectures were distorted. Pyknosis, karyorrhexis, and karyolysis were marked, and ductal lining epithelium showed scattered necrotic cells. The islets of Langerhans were largely unaffected. Mortality rate at the 22.5 and 20 mg/kg doses was 15-30% between 36 and 56 hr. Regenerative response was highly significant in those surviving past 56 hr. At lower doses acinar cell necrosis was not as pronounced, resulting in a few isolated necrotic cells between 24 and 48 hr. With the 22.5-20 mg/kg doses, maximal necrosis was reached by 48 hr. At 56 hr, numerous cells with round or oval nuclei and basophilic cytoplasm were seen which participated in formation of new acini. Numerous mitotic figures were apparent; these reached a peak by 60-66 hr. By 66-72 hr, acinar and lobular architecture resembled that of fetal pancreas. Regeneration was still in progress in some by 96-144 hr. In some cases it was incomplete, lobules being replaced by pseudoacinar or ductular tissue. Autoradiography showed marked increase in acinar cell labeling index at 56, 60, 66, 72, and 84 hr, compared with controls. About 8% of the nuclei were labeled at

graphy showed marked increase in acinar cell labeling index at 56, 60, 66, 72, and 84 hr, compared with

controls. About 8% of the nuclei were labeled at 56 hr, and reached a peak of 28%, declining after 72 hr. Labeled nuclei were more frequent in the ductal epithelium at 66 and 72 hr than in controls. At 48 and 56 hr (after the 22.5 mg/kg dose) serum amylase levels were markedly elevated, while that in the pancreatic homogenate was diminished. By 72 hr, serum levels returned to below normal, but tissue levels were not normal even after 144 hr. It is concluded that the 22.5 mg/kg 4-HAQO provides a good model of induced regeneration on which to investigate carcinogen uptake by regenerating pancreas.

0679 ORGAN SPECIFICITY OF ARYL HYDROCARBON HYDROXYLASE INDUCTION BY CIGARETTE SMOKE IN RATS AND MICE. (Eng.) Van Cantfort, J. (Lab. Clin. Chem. Univ. Liège, Belgium); Gielen, J. *Biochem. Pharmacol.* 24(11/12):1253-1256; 1975.

The mechanism of cigarette smoke induction of aryl hydrocarbon hydroxylase activity was investigated in the liver, lungs, and kidneys of male Sprague-Dawley rats and male C3H/Ico, C57Bl/6J, DBA/2J and AKR/Rho Ico mice. The rats and mice were allowed to inhale cigarette smoke during 15-min periods. The smoke leaving the cigarette was diluted by 15 volumes of air for rats and by 30 volumes for mice. When several inhalations were performed, they were administered at two hr intervals. The animals were always sacrificed four hr after the last inhalation. Fifteen min of smoke inhalation produced a significant aryl hydrocarbon hydroxylase induction in the lung and kidney of the rat. If successive inhalations were administered, two phenomena were observed: 1) up to four inhalations, each treatment induced the aryl hydrocarbon hydroxylase activity further; and 2) after the fourth inhalation, subsequent inhalations did not cause an augmentation of aryl hydrocarbon hydroxylase activity. The enzymatic activity decreased slowly after the sixth inhalation in the lung and in the kidney after the fourth. In mice submitted to three successive treatments, cigarette smoke induced the aryl hydrocarbon hydroxylase activity very significantly in the lungs of all the strains studied, while it did not affect the enzymatic activity in the livers of the same animals. The kidney aryl hydrocarbon hydroxylase activity was significantly induced in two strains of mice (C57Bl/6J and C3H/Ico) but was not modified in the other two strains. It is suggested that the inducibility of aryl hydrocarbon hydroxylase in the lungs of rats and mice (the latter belonging to either an inducible or a non-inducible strain) by cigarette smoke may be explained either by a lung specific receptor or by the administration route of the responsible agent. Several hypotheses are postulated to explain the differences in inducibility between the liver and the kidney and between the kidney in inducible and noninducible strains: 1) the kidney receptor is more sensitive than the liver receptor, 2) the inducing agent reaches a higher concentration in the lung compared to the kidney, and in the kidney compared to the other tissues, or 3) the responsible inducers might not be the same in the lung as in the kidney. This study demonstrates the great sensitivity of a lung enzyme, which is implicated in chemical carcinogenesis, to a common environmental agent.

0680 HUMAN PLACENTAL ARYL HYDROCARBON HYDROXYLASE: STUDIES WITH FLUORESCENCE HISTOCHEMISTRY. (Eng.) Gough, E. D. (Sch. Med., Univ. Washington, Seattle); Lowe, M. C.; Juchau, M. R. *J. Natl. Cancer Inst.* 54(4):819-824; 1975.

Cells from placental tissues possessing aryl hydrocarbon hydroxylase activity and which are subject to high inducibility by components present in cigarette smoke were investigated. Tissue sections from human placentas taken at term were studied after time-sequential incubations with benzo[*a*]pyrene and appropriate cofactors for mixed function oxidation. A solution (0.02 ml) containing benzo[*a*]pyrene (0.06 mg/ml) was applied to the tissue sections, the solvent was allowed to evaporate, and the sections placed into incubation vessels at 37 C. The reaction mixture contained 5.8×10^{-3} M NADPH, 1.1×10^{-4} M NADH, 5×10^{-3} M $MgCl_2$, 5×10^{-3} M KCl, and 3×10^{-2} M sodium phosphate buffer at pH 7.4 in a total volume of 10 ml. Sections were incubated for 15 min, one hr, and three hr. Control sections were incubated for the same period but in the following reaction mixtures: 1) those to which no pyridine nucleotides were added; 2) those to which no benzo[*a*]pyrene was added, and 3) those to which *p*-chloromercuribenzoate was added as an inhibitor. Studies were performed on placental tissues having low, intermediate, and high aryl hydrocarbon hydroxylase activities in assays of tissue homogenates. Fluorescence microscopy revealed that the enzymic reaction was most active in the syncytial trophoblast, although the fluorescence of hydroxylated metabolites also could be observed in other placental cell types. A comparison of sections from placentas with very low *versus* very high aryl hydrocarbon hydroxylase activities indicated that induction of the human placental enzyme system with polycyclic aromatic hydrocarbons also occurred primarily in the syncytium. These results indicate that fetal constituents of human placentas contain readily detectable aryl hydrocarbon hydroxylase activity, particularly if the mother has smoked cigarettes during her pregnancy. The implications of such findings in transplacental carcinogenesis, however, are not clear and further studies are suggested.

0681 EFFECTS OF CIGARETTE SMOKING ON ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN LUNGS AND TISSUES OF INBRED MICE. (Eng.) Abramson, R. K. (Univ. Kentucky Sch. Med., Lexington); Hutton, J. J. *Cancer Res.* 35(1):23-29; 1975.

The relationship between the inhalation of cigarette smoke and the aryl hydrocarbon hydroxylase (AHH) activity in the lungs and other tissues was examined in hydrocarbon-responsive and -nonresponsive strains of mice. Inbred strains of mice (females) were classified as aromatic hydrocarbon-responsive or -nonresponsive depending upon whether the parenteral administration of these substances increased the hepatic AHH a

tivity. Aromatic hydrocarbon responsiveness is controlled by genes at a small number of loci. Using 3-methylcholanthrene (i.p.) as the inducing agent, strains A/J, C3H/HeJ, and C57 BL/6J were classified as responsive, whereas strains AKR/J, DBA/2J, and SWR/J were nonresponsive. Animals from each strain were exposed daily for one day to four weeks to the smoke from six cigarettes (seven puffs/cigarette); controls were put through the same procedure without the cigarettes being lit (sham controls) or were left untreated (laboratory controls). Inhalation of cigarette smoke by both the responsive and nonresponsive mice induced AHH activity in the lung, but not in the liver, stomach, small intestine or kidney. The responsive strains had significantly higher levels of basal and induced AHH in the lung than did the hepatic nonresponsive strains. However, because of the especially low basal activity of AHH in the lungs of the nonresponsive strains, the ratio of AHH activity in the animals treated with cigarette smoke to that in the untreated animals was higher in the nonresponsive than in the responsive strains. AHH activity in the lung was fully induced within 6-12 hr after smoke inhalation and remained at the same level regardless of the number of days of treatment. AHH in the lung returned to basal levels within five days after the cessation of smoking. The results suggest that the genetic regulation of AHH activity in the lung has some influence on an individual's susceptibility to pulmonary neoplasms and bronchial inflammation.

0682 NON-ENZYMATIC AND MICROSOME-DEPENDENT BINDING OF POLYCYCLIC HYDROCARBONS TO DNA AND POLYNUCLEOTIDES. (Eng.) Grilli, S. (Istituto di Cancerologia, Universita di Bologna, via S. Giacomo 14, 40126 Bologna, Italy); Rocchi, P.; Prodi, G. *Chem. Biol. Interact.* 11(5):351-363; 1975.

The binding of [^{3}H]7,12-dimethylbenz(a)anthracene, [^{3}H]benzo(a)pyrene, and [^{3}H]3-methylcholanthrene to DNA or to polynucleotides *in vitro* was examined in the presence and absence of rat liver or human placental microsomes. The effects of temperature and light were investigated by incubating 4 mg DNA with 20 μCi of labeled dimethylbenz(a)anthracene, benzo(a)pyrene, or 3-methylcholanthrene, both in the dark and in light, and at 0 C and 50 C for 150 min in the dark. After ethanol precipitation, DNA was washed at least 50 times. For incubation in the presence (or absence) of microsomes, each reaction tube contained 0.15 ml 1 M buffer, 4 mg NADPH, 4 mg DNA or polynucleotide, 20 μCi labeled hydrocarbon (dissolved in 0.1 ml ethanol) and 1 ml microsomal suspension (omitted for nonenzymatic binding). After incubation for one hour, microsomes were removed by centrifugation, and DNA or polynucleotide was precipitated with ethanol. Precipitates were washed until no radioactivity was present in the extracts. The level of nonenzymatic binding was very high after 30 min of incubation and increased with time. Values for benzo(a)pyrene and 3-methylcholanthrene were higher in the presence of light, the opposite being observed for dimethylbenz(a)anthracene. Binding was temperature-dependent. In the absence of light, it followed the ascending order: benzo(a)pyrene, 3-methylcholanthrene, dimethylbenz(a)anthracene. The radioactivity bound to DNA in the absence of microsomes was higher than that bound to DNA incubated with either boiled, normal or induced microsomes, with the exception of benzo(a)pyrene. In the presence of induced microsomes from rat liver, all the polycyclic hydrocarbons bound to all the polynucleotides; the binding was higher than that observed in the absence of microsomes. Human placental microsomes were efficient in the case of benzo(a)pyrene except for poly G and, to a lesser extent, in the case of dimethylbenz(a)anthracene. Poly G seemed to be the least suitable polynucleotide to be used in the presence of both rat liver and human placental microsomes. Dialysis and enzymatic degradation assays showed that no more than 20% of total radioactivity was attributable to physical binding; the remaining 80% was neither dialyzed nor extracted by organic solvents. A large part (60-80%) of radioactivity overlapped the DNA peak after CsCL sedimentation indicating that this binding is covalent.

0683 COMPARISON OF THE CELLULAR DNA-BOUND PRODUCTS OF BENZO(*a*)PYRENE WITH THE PRODUCTS FORMED BY THE REACTION OF BENZO(*a*)PYRENE-4,5-OXIDE WITH DNA. (Eng.) Baird, W. M. (Inst. Cancer Res., London, England); Harvey, R. G.; Brookes, P. *Cancer Res.* 35(1):54-57; 1975.

To determine whether the "ultimate" carcinogens formed in cells from methyl-substituted polycyclic hydrocarbons differ from those formed from the non-methylated hydrocarbons, the products formed by the reaction of benzo(*a*)pyrene (BP)-epoxide (K-region epoxide) with DNA in aqueous solution were compared with those found in DNA isolated from cells which had been exposed to BP in culture. DNA isolated from mouse embryo cell cultures which had been treated with (^{3}H)BP was enzymically degraded to deoxyribonucleosides, and the hydrocarbon-deoxyribonucleoside products were isolated by chromatography on a Sephadex LH20 column eluted with a water:methanol gradient. The products were compared with those found in similar chromatograms of enzyme digests of DNA which had been reacted with BP-4,5-oxide in aqueous ethanol solution. The UV absorption profiles of the two sets of products were not identical. The results do not support the suggestion that the products formed in the DNA of BP-treated cells result from the metabolism of BP to a K-region epoxide and reaction of this compound with the cellular DNA. There may be several hydrocarbon-metabolizing pathways operating simultaneously in such cells, and the data suggest that the metabolic activation that results in polycyclic hydrocarbons becoming bound to the DNA of cells in culture may be more complicated than simply formation of a K-region epoxide.

0684 IMPORTANCE OF PHYSICAL PROPERTIES OF BENZO(*a*)PYRENE-FERRIC OXIDE MIXTURES IN LUNG TUMOR INDUCTION. (Eng.) Henry, M. C. (Life Sci. Div., IIT Res. Inst., Chicago, Ill.); Port, C. D.; Kaufman, D. G. *Cancer Res.* 35(1):207-217; 1975.

The carcinogenic properties of three benzo(a)pyrene (BP)-ferric oxide mixtures prepared by different

methods were compared in randombred male and female Syrian golden hamsters. The animals were given 30 wkly intratracheal instillations of mixtures containing 1% ferric oxide and 1% BP suspended in 0.5% gelatin in sterile 0.9% NaCl solution; controls were given 1% BP in gelatin-NaCl solution or the gelatin-NaCl vehicle alone. For coated BP-ferric oxide preparations, BP was attached to the ferric oxide dust by nucleation of the carcinogen on the particle at low temperature. The ground BP-ferric oxide mixture was prepared by mixing equal weights of BP and dust in a mullite mortar and grinding. The third mixture was prepared by adding the dry dust to a BP-gelatin suspension, to give an equal weight of carcinogen and ferric oxide. The animals treated with the coated preparation, which contained large aggregates of BP and ferric oxide, showed an earlier onset and higher incidence of respiratory tract tumors than the animals given the ground mixture, which contained smaller aggregates. With the third preparation, there was a low tumor incidence, similar to that seen in controls treated with BP in gelatin without ferric oxide. The greatest number of tumors was found in the trachea, the predominant histologic type being the squamous carcinoma. Squamous tumors as well as adenocarcinomas were observed in smaller numbers in the bronchi and lung parenchyma. The results indicate that for this model system of respiratory carcinogenesis, the physical attachment of BP to the carrier dust is necessary for a high tumor yield.

0685 MUTAGENIC AND CYTOTOXIC ACTIVITY OF BENZO[*a*]PYRENE 4,5-,7,8-, AND 9,10-OXIDES AND THE SIX CORRESPONDING PHENOLS. (Eng.) Wood, A. W. (Dept. Biochemistry and Drug Metabolism, Hoffman-La Roche Inc., Nutley, N.J. 07110); Goode, R. L.; Chang, R. L.; Levin, W.; Conney, A. H.; Yagi, H.; Dansette, P. M.; Jerina, D. M. *Proc. Natl. Acad. Sci. USA* 72(8):3176-3180; 1975.

The benzo[*a*]pyrene (BP) 4,5-, 7,8-, and 9,10-oxides and the six corresponding phenols (4-, 5-, 7-, 8-, 9-, and 10-hydroxybenzo[*a*]pyrene (HOBP) have been tested for mutagenic and cytotoxic activity in bacteria and in a mammalian cell culture system. BP 4,5-oxide (K-region) was highly mutagenic in two histidine-dependent strains (TA1537 and TA1538) of *Salmonella typhimurium* which detect frameshift mutagens. In contrast, BP 7,8- and 9,10-oxides were less than 1% as mutagenic as the 4,5-oxide. BP 7,8- and 9,10-oxides were unstable in aqueous media, whereas the 4,5-oxide was stable for several hr. This difference in stability could not account for the different mutagenic activities of the three arene oxides. The BP oxides were inactive in a strain (TA1535) that is reverted by base pair mutagens such as *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine or in a strain (TA1536) that detects frameshift mutagens similar to the acridine half-mustard ICR-191. BP and the six phenols were all stable in aqueous media, but they had little or no mutagenic activity in any of the four *Salmonella* strains. Conversion of 8-azaguanine-sensitive Chinese hamster V79 cells to 8-azaguanine-resistant variants was increased by BP 4,5-oxide, whereas the 9,10-oxide was considerably less active. BP and the other derivatives had little or no effect. BP 4,5-oxide was more cytotoxic to the Chinese hamster V79 cells than the 7,8- and 9,10-oxides, while 8-HOBP was the most cytotoxic of the six phenols.

0686 THE RELATIONSHIP OF CELLULAR METABOLISM OF BENZO(a)PYRENE TO THE INDUCTION OF CYTOTOXICITY AND CELL TRANSFORMATION IN FETAL HAMSTER CELLS *IN VITRO*. (Eng.) Lubet, R. A. (Southwestern Med. Sch., Dallas, Tex.); Turner-Lubet, M.; Whitson, G. L. *Eur. J. Cancer* 11(3):139-144; 1975.

The relationship of the cellular metabolism of benzo(a)pyrene [B(a)P] to the induction of cytotoxicity and cell transformation in fetal Syrian golden hamster cells was investigated *in vivo*. Secondary cultures of 2 to 4 day-old fetal hamster cells were incubated for 24 hr and then 0.01 or 0.02 ml of B(a)P in dimethylformamide or B(a)P plus inhibitor, benzanthracene (10 μg/ml), or 7,8-benzoflavone (5 μg/ml) was added to the cells. Metabolism was measured using ^{3}H-B(a)P. Both benzanthracene and 7,8-benzoflavone reduced the toxicity of B(a)P; benzanthracene reduced the toxicity of B(a)P by 25-30%, while reducing the overall metabolism of B(a)P by 35-40% at 48 hr. 7,8-Benzoflavone reduced B(a)P-mediated toxicity 65-75%, while causing a 70-80% decrease in overall B(a)P metabolism. These findings support the view that the toxicity of B(a)P parallels the ability of the treated cells to metabolize it. At 5 μg B(a)P/ml, approximately 0.16% of the plated cells grew into colonies which showed neoplastic transformation according to such criteria as lack of contact inhibition and random growth. Addition of benzanthracene or benzoflavone either prior to or in conjunction with B(a)P resulted in no significant enhancement in the percentage of morphologically transformed cells. The results confirmed that the toxicity of B(a)P is closely linked to its overall metabolism, while transformation does not directly relate to the overall B(a)P metabolism.

0687 METABOLIC PATHWAYS OF 7,12-DIMETHYLBENZ[*a*]-ANTHRACENE IN HEPATIC MICROSOMES. (Eng.) Yang, S. K. (Arthur Amos Noyes Lab. of Chemical Physics, California Inst. of Technology, Pasadena, Calif. 91125); Dower, W. V. *Proc. Natl. Acad. Sci. USA* 72(7):2601-2605; 1975.

High pressure liquid chromatography has enabled quantitative analysis of the *in vitro* metabolism of 7,12-dimethylbenz[*a*]anthracene (DMBA), 7-methyl-12-hydroxymethylbenz[*a*]anthracene (7-M-12-OHMBA), 7-hydroxymethyl-12-methylbenz[*a*]anthracene (7-OHM-12-MBA), and 7,12-dihydroxymethylbenz[*a*]anthracene (DMBA-8,9-diOHMBA) by 3-methylcholanthrene-induced and control rat liver microsomes. The following previously unrecognized metabolites have been tentatively identified: 5,6-dihydro-5,6-dihydroxy-7-methyl-12-hydroxymethylbenz[*a*]anthracene, 3-hydroxy-7,12-dihydrodihydroxymethylbenz[*a*]anthracene, 4-hydroxy-7,12-dihydrodihydroxymethylbenz[*a*]anthracene, and 8,9-dihydro-8,9-dihydroxy-7,12-dihydroxymethylbenz[*a*]anthracene. The epoxide hydratase inhibitor 1,2-epoxy-3,3,3-trichloropropane was found to eliminate all dihydrodiol formation and markedly inhibit the formation of several dimethylbenzanthracene metabolites. It is pro-

posed that the tentatively identified 3-hydroxy and 4-hydroxy derivatives are formed by an enzymatic mechanism that does not involve epoxides as intermediates. The metabolic pathways of DMBA in hepatic microsomal enzymes are proposed.

0688 TRANSPLANTATION OF ADENOCARCINOMAS OF THE COLON IN MICE. (Eng.) Double, J. A. (Sch. Med., Leeds, England); Ball, C. R.; Cowen, P. N. *J. Natl. Cancer Inst.* 54(1):271-275; 1975.

A relatively stable, well-differentiated model for colorectal cancer is described. Five serially s.c. transplantable tumor lines were established from colon tumors induced by 1,2-dimethylhydrazine treatment in NMRI mice. All of the tumors were adenocarcinomas varying in degree of differentiation and mucin production, and during up to six transplant generations there was no evidence of dedifferentiation or change in growth rates. Spontaneous necrosis was not common or extensive enough to obscure the results of therapy. Preliminary results indicate a general insensitivity to the best chemotherapeutic agents available for gastrointestinal cancer in man, but each line had a characteristic sensitivity to a group of drugs.

0689 7,12-DMBA INDUCED RAT MAMMARY TUMOUR STUDIED FOR HORMONAL RESPONSIVENESS *IN VITRO*. 2. ORGAN CULTURES. (Eng.) Aspegren, K. (Tornblad Inst., Univ. Lund, Sweden). *Acta Pathol. Microbiol. Scand.* [*A*] 83(1):37-50; 1975.

The effect of various concentrations of testosterone, progesterone and 17-β-estradiol on cell survival and multiplication was measured in 7,12-dimethylbenz(a) anthracene (DMBA)-induced rat mammary tumors and sarcomas and in virus-induced sarcomas. Mammary tumors were induced in female outbred Sprague-Dawley rats by intragastric instillation of 20 mg DMBA; sarcomas were induced in similar rats by s.c. injection in the scapular region of 2 mg DMBA in dimethyl sulfoxide and the virus sarcomas were one adeno-, one polyoma- and one Rous-virus induced tumor, each serially transplanted for at least 12 generations before use. Five mammary tumors and six sarcomas were cultured in testosterone in concentrations of 0.5, 5, and 50 μg/ml. Four mammary tumors showed a similar dose-dependent response. With 50 μg/ml, they were significantly inhibited at 24 hr. This response decreased with increasing time. One virus sarcoma was not significantly affected by any concentration at any culture times. Another was stimulated by 5 μg/ml. There was no consistent variation of response with time. Five mammary tumors were tested with progesterone at concentrations of 0.8, 8, and 80 μg/ml. All tumors were inhibited by 80 μg/ml; the response increased with increasing time. One sarcoma was not significantly affected by the treatment except by 80 μg/ml at 24 hr; three others were not affected at lower concentrations, but were strongly inhibited at 80 μg/ml and even more with increasing time. Five mammary tumors, three DMBA-induced sarcomas, and three virus-induced and serially transplanted sarcomas were incubated in 17-β-estradiol at concentrations of 0.02, 0.2, and 20 μg/ml. The analysis of interaction between tumor and hormone concentration was highly significant at all concentrations for the sarcoma group. The mammary tumors showed no significant interaction at 0.02-2 μg/ml but a significant interaction at 20 μg/ml. The results demonstrate *in vitro* reactions to added hormones of the two types of tumor.

0690 POLY(rA):OLIGO(dT)-DIRECTED DNA POLYMERASE ACTIVITY IN CARCINOGEN INDUCED RAT MAMMARY TUMORS AND IN NORMAL TISSUES. (Eng.) Libertini, L. J. (Chem. Biodyn. Lab., Lawrence Berkeley Lab., Univ. California); Adams, J. *Biochem. Biophys. Res. Commun.* 64(1):136-143; 1975.

DNA polymerases of carcinogen-induced rat mammary tumors and their synthetic template activity were studied and compared to the activity of polymerases found in RNA tumor viruses. Mammary tumors were obtained from Sprague-Dawley rats which had been treated with 7,12-dimethylbenz(a)anthracene. After subcellular fractionation of the tumor tissue, the microsome fraction contained 90% of the activity on the synthetic template, poly(rA):oligo(dT). The polymerase contained in this fraction was also active on poly(rC):oligo(dG), but was inactive on poly(dA): oligo(dT). Its molecular weight was 105,000 daltons. This polymerase resembles those from RNA tumor viruses in its synthetic template activity and molecular weight. A similar polymerase was also found in lactating normal rat mammary tissues. The authors conclude that it is unclear whether the polymerase they describe is a virus-related polypeptide or a normal cellular polymerase with an unknown function.

0691 NERVOUS SYSTEM ANTIGEN-2 (NS-2), AN ANTIGENIC CELL SURFACE COMPONENT EXPRESSED ON A MURINE GLIOBLASTOMA. (Eng.) Schachner, M. (Harvard Med. Sch., Boston, Mass.); Carnow, T. B. *Brain Res.* 88(2):394-402, 1975.

Glioblastoma induced with methylcholanthrene in the C57BL/6J mouse strains was used to observe the nervous system antigen-2 (NS-2), an antigen cell surface component. To detect any immunization processes, female rabbits were injected s.c. and intradermally with tumor cells washed with Earle's Balanced Salt Solution in complete Freund's adjuvant. Antiserum was obtained from pretreated rats after bleeding them for seven days. Cytotoxicity was measured serologically to detect cell surface antigens. Cell viability was carried out by dispersing cells from solid tumor masses in high concentrations of trypsin and DNase I treatment. Antiserum activity was determined by calculating the percentage of dead cells in a total cell suspension. Cross-reactivity of NS-2 antigen between glioblastoma as target indicator cells was measured by test absorption of anti-NS-2 antiserum with particulate fractions of tissue homogenates. The blocking capacity of anti-NS-1 antibody for attachment of anti-NS-2 antibody to brain tissue homogenate was measured. Nervous system antigen-2 (NS-2) expressed on murine glioblastoma exhibited a rabbit antiglioblastoma cytotoxic titer of 1:500 on glioblastoma, 1:400 on C57BL/6j thymocytes and a titer of 1:100 on C57BL/6j lymph nodes. Viable glioblasto-

ma cells showed a ring fluorescence of 95% while 95% of the C57BL/6J lymph node cells or thrombocytes showed no fluorescence. Cross-reactivity was observed only in glioblastoma and brain tissue. Mouse tumors of non-neural origin showed no absorptive capacity for anti-NS-2 antiserum, but absorptive capacity was expressed in mouse tumors of putative glial cell origin. NS-2 was not observed in C57BL/6J mouse brain tissue until the sixth postnatal day and increased linearly until it reached adulthood. All anti-NS-2 activities were removed from the antiserum of brain tissue absorption, with liver slightly decreasing the antiserum activity. Cell types found in the isocortex (myelinated fiber) and diencephalon expressed a greater quantity of NS-2. The results indicate that the association of NS-2 with myelin fibers clearly distinguishes this system from NS-1. It is also concluded that cross-reactivity occurred only in glioblastoma and brain tissue.

0692 THE ROLE OF CELL DIVISION IN THE MALIGNANT TRANSFORMATION OF MOUSE CELLS TREATED WITH 3-METHYLCHOLANTHRENE. (Eng.) Kakunaga, T. (Res. Inst. Microbial Diseases, Osaka Univ., Suita, Osaka, Japan). *Cancer Res.* 35(7):1637-1642; 1975.

The requirement for cell division in the malignant transformation of A31-714 cells (a subclone derived from BALB/3T3) by 3-methylcholanthrene was investigated using the property of its high susceptibility to density-dependent inhibition of cell growth. Treatment with 3-methylcholanthrene did not induce transformation in a nongrowing population, unless they were returned to the growing state soon after treatment. This resulted in a high transformation rate near maximum level. About four cell generations were necessary for the development of cell transformation after treatment with 3-methylcholanthrene. Cells that were kept in a nongrowing state after carcinogen treatment rapidly lost their ability to express transformation even when they were subsequently returned to a growing state. Cells that were allowed one cell division soon after carcinogen treatment, however, retained their ability to produce transformed foci even after being kept in the nongrowing state thereafter. These results suggest that one cell generation is required for the fixation of transformation and that several additional cell generations are required for the expression of the transformed state.

0693 MULTIPLE FORMS OF CYTOCHROME P-450 IN PHENOBARBITOL- AND 3-METHYLCHOLANTHRENE-TREATED RATS. (Eng.) Ryan, D. (Dep. Biochem. Drug Metab., Hoffman-La Roche Inc., Nutley, N. J.); Lu, A. Y. H.; West, S.; Levin, W. *J. Biol. Chem.* 250(6):2157-2163; 1975.

Multiple forms of liver microsomal cytochrome P-450 isolated from immature male rats pretreated with phenobarbital or 3-methylcholanthrene (3-MC) are described. A fraction of low specific content (Fraction A, 1.7-4.0 mM of cytochrome P-450/mg of protein) and a substantially purified fraction (Fraction B, 9.0-11.0 mM of cytochrome P-450/mg of protein) were obtained by DEAE-cellulose chromatography of a partially-purified cytochrome P-450 preparation in the presence of Emulgen 911. Shifts in the absorption maxima in the CO-reduced and ethyl isocyanide difference spectra were observed in the fractions derived from the 3-MC-treated rats; the fractions derived from the phenobarbital-treated animals exhibited different 455:430 ratios and pH intercepts in the ethyl isocyanide difference spectra. The absolute oxidized spectra and *n*-octylamine binding spectra at room temperature and the EPR data at the temperature of liquid helium showed all the fractions, except Fraction A from the 3-MC-treated rats, to be low spin ferric hemeproteins. The A hemeprotein fractions from both groups of rats had poor catalytic activity for the metabolism of benzphetamine and 3,4-benzo(*a*)-pyrene in comparison to the B hemeprotein fractions; this may have been due to the presence of a high concentration of Emulgen 911 in the A fractions. However, the presence of Emulgen 911 could not account for the spectral differences among the fractions. The data therefore indicate the presence of two forms of cytochrome P-450 in the livers of phenobarbital-treated rats and the presence of two forms of cytochrome P-448 in the livers of 3-MC-treated rats.

0694 INTERMOLECULAR LINKING AND FRAGMENTATION OF DNA BY β-PROPIOLACTONE, A MONOALKYLATING CARCINOGEN. (Eng.) Kubinski, H. (Univ. Wisconsin Med. Sch., Madison); Szybalski, E. H. *Chem. Biol. Interact.* 10(1):41-55; 1975.

The effects of β-propiolactone (BPL) on purified *Escherichia coli* DNA were studied. ^{32}P-labeled bacterial DNA was incubated at 40 C with 0.5% BPL for varying periods of time, after which the DNA was studied by velocity sedimentation in sucrose gradients, by gel electrophoresis, ultraviolet spectrophotometry, and electron microscopy. Brief (15 min) exposure to BPL increased the sedimentation rate of the purified bacterial DNA in both neutral and alkaline sucrose gradients. However, when electrophoresed in polyacrylamide-agarose gels, the treated DNA moved ahead of the nontreated control. After longer periods of incubation (60 min), the DNA could be separated into two new fractions, the more prominent one being recovered as a heterogeneous peak in the 6-8S area of the gradient, and the remainder sedimenting very rapidly and being retained at the bottom of the tube after fraction collection. In acrylamide-agarose gels, the first fraction was again recovered in the 6-8 S area, while the second fraction failed to enter the gel at all. The DNA at this stage was hyperchromic in UV light, which indicated that as much as 20% of it may have been denatured. Coliphage λDNA treated briefly (1-5 min) with 2% BPL and spread in a protein monolayer appeared under the electron microscope as a rigid, extended molecule which was up to 15% longer than the untreated control DNA and usually occurred in compact, folded configurations suggestive of intra molecular linking. After longer exposure (5-10 min), localized denaturation associated with single-strand breaks was observed. After 30 min, the DNA appeared in the form of branched, interconnected networks of single-, double-, and possibly multi-stranded filaments; these networks increased in complexity and aggregate size with continued exposure to BPL. It

is suggested that exposure to relatively low concentrations of BPL *in vivo* might produce nonlethal genetic recombinations or chromosomal rearrangements, some of which might lead to mutagenesis or carcinogenesis.

0695 JUNCTIONAL SPECIALIZATION IN ESTROGEN-INDUCED RENAL ADENOCARCINOMAS OF THE GOLDEN HAMSTER. (Eng.) Letourneau, R. J. (Beth Israel Hosp., Boston, Mass.); Li, J. J.; Rosen, S.; Villee, C. A. *Cancer Res.* 35(1):6-10; 1975.

The junctional specialization found in estrogen-dependent renal adenocarcinomas in the golden hamster was correlated with that in other tissues sensitive to steroid hormones. Diethylstilbestrol or estradiol-17β pellets were implanted subpannicularly every three months into mature castrated male Syrian hamsters. Normal and tumorous kidneys were removed after the appearance of tumor nodules (8-10 months after hormone treatment), the hormone pellets being removed 7-12 days prior to sacrifice in some animals. The tissues were impregnated with 2% lanthanum and examined by electron microscopy. Distinct membrane specialization in the form of gap junctions was seen in the renal tumors, similar junctional differences being seen in the proximal tubules of the normal kidneys. Although both cell types possessed gap junctions, the neoplastic cells had, in addition, a cytoplasmic configuration of gap-junctional membrane (annular nexuses) not found in the kidneys of untreated or estrogenized hamsters or in the nontumorous kidneys adjacent to the adenocarcinomas. These unique junctional structures were present in the abdominal metastases as well as in the primary lesions and appeared to be consistent characteristics of these tumors. When the renal tumors were deprived of estrogen for 7-12 days, the number of annular gap junctions appeared to decrease. The results suggest that the presence of the unique gap-junctional membrane in the renal tumors may be related to their estrogen dependency.

0696 *IN VITRO* REACTION OF β-PROPIOLACTONE AND γ-BUTYROLACTONE WITH GLUTATHIONE AND CYSTEINE. (Eng.) Dijkstra, J. (S. Afr. Counc. Sci. Ind. Res., Pretoria). *Chem. Biol. Interact.* 10(2): 115-121; 1975.

The *in vitro* reaction of the carcinogen, β-propiolactone (BPL), and the non-carcinogen, γ-butyrolactone (GBL), with glutathione (GSH) and cysteine (CySH) was examined by ultraviolet spectrophotometry at 233 nm to study the nature of the reaction products. The reaction mixtures contained 0.05 ml 0.052 M GSH or CySH in 1 M potassium phosphate (pH 6.6), and 0.05 ml 0.05 M BPL or GBL in absolute ethanol. The blanks contained the same ingredients except that the sulfhydryl compound was replaced by buffer or the lactone by absolute ethanol. The hydroxamic acid test for thioester was done on the mixture of 1 mg of lactone and 10 mg GSH in 0.3 ml 0.1 M sodium acetate (pH 6.5) after 60 min at ambient temperature. After formation of thioester, the reaction of GSH and BPL was examined chromatographically. The reaction of BPL and GSH resulted in a 0.24 U increase in absorbance at 233 nm after 141-153 min. After addition of NH_2OH, the absorbance decreased by 0.20 U after 11-15 min. The reaction of GSH and BPL yielded two products chromatographically, one with an R_f of 0.64 on silica and 0.33 on paper (identified as thioester by the hydroxamic acid test) and another with an R_f of 0.55 on silica and 0.19 on paper (identified as thioether by iodoplatinate and sulphoxide tests). GBL and GSH or CySH did not form thioester since absorbance at 233 nm remained constant. The reaction of BPL with GSH produces thioester and thioether. GBL did not acylate the sulphydryl group of either GSH or CySH. The reaction products of GSH and BPL are being isolated to elucidate their structure and to evaluate their carcinogenetic properties.

0697 OVARIAN CYSTADENOFIBROMA: A CONSIDERATION OF THE ROLE OF ESTROGEN IN ITS PATHOGENESIS. (Eng.) Papadaki, L. (Middlesex Hosp. Med. Sch., Univ. London, England); Beilby, J. O. W. *Am. J. Obstet. Gynecol.* 121(4):501-512; 1975.

A series of 39 ovarian cystadenofibromas obtained over a period of seven yr from 35 patients (29-82-yr-old) were studied by light and electron microscopy to investigate the role of estrogen in the pathogenesis of this tumor. The accompanying fallopian tube was also studied in 24 patients and the endometrium was studied in 18. The tumor epithelium was tubal in type and, at the ultrastructural level, was similar to that of the normal fallopian tube during estrogenic stimulation. The appearance of the stomal fibroblasts in the connective tissue component of the tumors was also consistent with an estrogenic influence. The effects of estrogen on the morphology of the fallopian tube involved: an increase in the number of polysomes and in the quantity of rough-surfaced endoplasmic reticulum, with distension of the cisternae; an augmentation of the Golgi complex; an increase in the number of mitochondria; proliferation of the cilia in one cell type and formation of secretory granules in the other; an increase in pinocytotic activity; and the appearance of lysosomes and autophagic vacuoles. With the exception of secretory granules, all of these features were seen in the epithelium of the cystadenofibroma. An estrogenic influence on the pathogenesis of the cystadenofibroma is clearly implicated; it is suggested that the source of estrogen responsible for both the epithelial differentiation and fibrous proliferation may be found in functional steroidogenic cells present in the stroma of the neoplasm.

0698 LATE EFFECTS IN THE VAGINAL AND CERVICAL EPITHELIA AFTER INJECTIONS OF DIETHYLSTILBESTROL INTO NEONATAL MICE. (Eng.) Forsberg, J. G. (Inst. Anat., Bergen, Norway). *Am. J. Obstet. Gynecol.* 121(1):101-104; 1975.

The late effects of neonatally-injected diethylstilbestrol on the vaginal and cervical epithelium of NMRI mice were studied. Diethylstilbestrol (5 μg) was injected s.c. into three mice within 24 hr of birth, the injection being repeated four times at

24-hr intervals. The animals were then killed at 13 months of age, and the serially sectioned uterine cervix and upper vaginal region were examined histologically. The study showed extensive adenosis comprising most of the cervical wall, and in some regions the glandular epithelium had undergone epidermatization. There was suggestive evidence of cancerous development in both the glandular epithelium and in the epidermized regions. In the vaginal region, the squamous epithelium showed hyperplasia, but there were also glandular ducts penetrating far into the subepithelial tissue. The results suggest that the estrogenic effect on the cervicovaginal epithelium of the neonatal mouse during its differentiation phase of development might serve as a pathogenic model for the development of adenocarcinomas in young women whose mothers were given diethylstilbestrol during pregnancy.

0699 TESTOSTERONE METABOLISM IN BENIGN AND MALIGNANT BREAST LESIONS. (Eng.) Rose, L. I. (Peter Bent Brigham Hosp., 721 Huntington Ave., Boston, Mass. 02115); Underwood, R. H.; Dunning, M. T.; Williams, G. H.; Pinkus, G. S. *Cancer* 36(2): 399-403; 1975.

Tissues from a variety of breast lesions were incubated with ^{14}C-testosterone (17β-hydroxy-4-androsten-3-one) to compare the extent of formation of 5α-dihydrotestosterone and androstenedione from testosterone. Specimens from six patients with fibroadenoma, seven with adenocarcinoma, ten with fibrocystic disease, and from ten individuals with normal breasts were used. Samples of breast tissues were obtained at the time of surgery, minced, and incubated (37 C, 95% O_2/5% CO_2 atmosphere, pH 7.4) with buffer containing 2 mg dextrose and 0.05-0.15 µCi (0.89-2.67 nM) ^{14}C-testosterone. After one hour, methanol (8 ml) was added to stop the steroid metabolism. The samples were diluted with ethanol to 10 ml, and a 1% aliquot was removed; 15,000 dpm ^{3}H-5α-dihydrotestosterone and ^{3}H-androstenedione were added to the remaining 99% of every sample. The doubly labeled 5α-dihydrotestosterone and androstenedione were extracted and purified to constant ^{3}H/^{14}C ratio by derivative formation and successive paper chromatography in several different solvent systems. Eluates from the final paper chromatography of ^{3}H/^{14}C 5α-dihydrotestosterone acetate and ^{3}H/^{14}C androstenedione were mixed with 70 mg of standard 5α-dihydrotestosterone acetate and androstenedione, respectively and crystalized and recrystallized from ethanol. After each crystallization, an aliquot was dissolved in ethanol, and the mass was determined by measuring the optical density at 240 µm in a UV spectrometer for androstenedione, and by weighing for 5α-dihydrotesterone acetate. All normal breast tissue exhibited formation of ^{14}C-androstenedione from ^{14}C-testosterone, but no conversion to ^{14}C-5α-dihydrotestosterone was found. All fibroadenoma tissues showed a greater percentage conversion of ^{14}C-testosterone to ^{14}C-androstenedione than did any normal sample; 4 of 7 fibroadenomas from three patients showed conversion of ^{14}C-testosterone to ^{14}C-5α-dihydrotestosterone. Tissues from four patients with fibrocystic disease showed a conversion of ^{14}C-testosterone to ^{14}C-androstenedione that was greater than that found in normal breast tissue; tissues from two of these patients also showed conversion of ^{14}C-testosterone to ^{14}C-5α-dihydrotestosterone. All but one of the tissues from the patients with adenocarcinoma showed a percentage conversion to ^{14}C-androstenedione that did not differ significantly from normal breast tissues. Contrary to a previously published report, no sample of adenocarcinoma tissue showed any conversion to ^{14}C-5α-dihydrotestosterone. The data suggest that formation of 5α-dihydrotestosterone is a predominant metabolic pathway in fibroadenoma.

0700 A NEW CARCINOGEN? (PRELIMINARY REPORT). (Eng.) Shafer, N. (New York Medical Coll., N.Y.); Shafer, R. *Curr. Ther. Res.* 17(4):407; 1975.

The possibility that hair dyes and bleaches are carcinogenic is hypothesized. A review of case histories of breast cancer patients over a period of years showed that 87% of them had been longtime users of hair coloring agents. Although the packages of the dyes and bleaches do not list their contents, they do contain some or all of the following: ammonium hydroxide, isopropyl alcohol, ethoxydiglycol, oleic acid, glycerin, propylene glycol, octyphenoxy polyethoxy ethanol, nonoxynol 4, cocamide DEA, free acid of complex organic phosphate ester, perfume, *p*-phenylenediamine, resorcinol, 4-methoxy-*m*-phenylenediamine sulfate, 4-nitro-*p*-phenylenediamine, 2-nitro-*p*-phenylenediamine, hydroquinone, sodium sulfite, toluene-2, 5-diamine sulfate, *o*-amino-phenol, *m*-aminophenol, 4-amino-2-nitrophenol, acid yellow 3, EDTA, erythorbic acid, and thiosalicyclic acid. Many chemicals, including hair coloring products, can penetrate the skin, particularly if it is irritated. Hair colorants are applied to the hair and permitted to remain for a considerable period of time to allow the color to take. Thus, there is ample opportunity for absorption by the skin of the scalp and contiguous areas, particularly if it is irritated. The repeated use of any of these chemicals known to be carcinogenic might trigger cancer in susceptible persons. Studies are being conducted on laboratory animals to test this hypothesis.

0701 MECHANISM OF CELL ENTRY AND TOXICITY OF AN AFFINITY-PURIFIED LECTIN FROM *RICINUS COMMUNIS* AND ITS DIFFERENTIAL EFFECTS ON NORMAL AND VIRUS-TRANSFORMED FIBROBLASTS. (Eng.) Nicolson, G. L. (Salk Inst. Biol. Stud., San Diego, Calif.): Lacorbiere, M.; Hunter, T. R. *Cancer Res.* 35(1):144-155; 1975.

An affinity-purified plant lectin from *Ricinus communis* (RCA_{II}) was used to investigate the mechanism of *Ricinus communis* lectin toxicity and also its effects on normal and simian virus 40 (SV40)-transformed cells. Cells were grown to subconfluency, treated with lectin solution for various times at 37 C, incubated with ^{3}H-leucine (3 µCi/ml) for one hr, and protein was precipitated. RCA_{II} suppressed cell protein synthesis in the transformed line at lower concentrations (1/50 and 1/100) compared to

the 3T3 line; these effects were blocked by the RCA_{II} inhibitors, D-galactose or lactose. RNA and DNA synthesis and L-leucine transport were relatively unaffected by RCA_{II} concentrations (>1 μg/ml) that completely suppressed protein synthesis in both cell lines. The kinetics of ^{125}I-RCA_{II} binding to intact cells (saturation in approximately 5-10 min, even at 4 C) compared to the time required to inhibit cell protein synthesis (>80% inhibition in approximately 60 min at 37 C) indicated that events subsequent to cell binding were necessary for the action of RCA_{II}. That RCA_{II} acts directly on protein synthesis after cell entry was confirmed with rabbit reticulocyte and mouse Krebs II ascites S30 cell-free protein synthesis systems. Concentrations of RCA_{II} (0.1-1 μg/ml) that inhibit cell protein synthesis in 90 min act within 1-3 min to suppress cell-free protein synthesis. The saccharide prevention of RCA_{II} inhibition of cell, but not cell-free, protein synthesis indicated that different sites may be involved in cell binding and inhibition of protein synthesis. The results suggest that RCA_{II}-mediated toxicity occurs by the following sequence of events: 1) cell surface binding; 2) lectin-induced surface clustering of RCA_{II} receptors; 3) endocytosis of RCA_{II}; 4) release of RCA_{II} from inside endocytotic vesicles into the cell cytoplasm; and 5) direct RCA_{II} interaction and inactivation of protein synthesis.

0702 HEPATOCARCINOGENESIS BY DIETHYLNITROSAMINE IN RATS FED HIGH DIETARY LEVELS OF LIPOTROPES. (Eng.) Poirier, L. A. (Notre-Dame Hosp., Montreal, Canada). *J. Natl. Cancer Inst.* 54(1):137-140; 1975.

The effects of high dietary levels of lipotropes on the carcinogenic activity of diethylnitrosamine (DENA) in young-adult Wistar rats were studied. All animals given DENA, with or without a dietary supplement, developed hepatocellular carcinomas. The mean survival times of all groups dying with hepatocellular carcinomas were determined. Choline, betaine and folic acid exerted no significant effect on the mean survival times. In rats receiving approximately 2.0 mg DENA/day, methionine administration led to a slight but significant increase in the mean survival time, whereas vitamin B_{12} significantly lowered the survival time. Neither substance altered the mean survival times of rats given 1.0 mg DENA/day. Ethanolamine decreased the mean survival times of rats given 1.0 mg DENA/day, but had no effect on animals receiving 2.0 mg daily. Demethylthetin, methotrexate, lecithin, and cephalin exerted no effect on the carcinogenic activity of DENA. The administration of dimethylthetin and betaine along with DENA led to an increase in liver weights in animals dying of hepatocellular carcinomas, when compared to liver weights of rats treated with DENA alone. It is concluded that, with the dietary mixtures used, the administration of lower doses of DENA would not result in greater effects of the lipotropes on tumor production. However, the use of lipotrope-deficient diets would probably amplify the differences in tumor incidence or mean survival times observed between animals treated with carcinogens alone and those treated with carcinogen plus lipotrope.

0703 REPRESSION OF DIMETHYLNITROSAMINE-DEMETHYLASE BY TYPICAL INDUCERS OF MICROSOMAL MIXED-FUNCTION OXIDASES. (Eng.) Arcos, J. C. (U.S. Public Health Serv. Hosp., New Orleans, La.); Bryant, G. M.; Venkatesan, N.; Argus, M. F. *Biochem. Pharmacol.* 24(16):1544-1547; 1975.

The repression of dimethylnitrosamine-demethylase activity by inducers of microsomal mixed-function oxidases was investigated in the rat. Weanling male Sprague-Dawley rats were pretreated with the test compounds as follows: (1) 3-methylcholanthrene at 40 mg/kg, benzo[*a*]pyrene and β-naphthoflavone at levels equimolar to those for 3-methylcholanthrene, and aminoacetonitrile at 200 mg/kg, all injected ip six hours before sacrifice, (2) pregnenolone 16α-carbonitrile given in four ip injections at 60 mg/kg at 43, 31, 19, and 6 hr prior to sacrifice, (3) phenobarbital given daily for four consecutive days ip at 80 mg/kg, the last injection being 24 hr before sacrifice, and (4) aroclor 1254 (a polychlorinated biphenyl mixture) in a single ip injection at 500 mg/kg, four days prior to sacrifice. With the exception of benzo[*a*]pyrene and aminoacetoninitrile, all compounds resulted in a significant increase in liver weight. Phenobarbital and Aroclor 1254 were the only two agents that brought about liver hypertrophy coincident with a statistically significant change in the amount of microsomes. With Aroclor 1254, liver hypertrophy was accompanied by a decrease in microsomal protein. An increase in liver weight together with substantial proliferation of the endoplasmic reticulum was seen after pretreatment with phenobarbital. All test compounds brought about substantial and significant decreases in enzyme activity when the enzyme activity was expressed in terms of nM HCHO produced/hr/mg microsomal protein, with aminoacetonitrile being the most potent and phenobarbital the least potent. All compounds except phenobarbital substantially repressed dimethylnitrosamine demethylase activity when expressed per gram of liver; in the case of phenobarbital, there was no change because the decrease of enzyme activity per milligram microsomal protein is compensated for by an increase of microsomal yield of similar magnitude. It is suggested that the discrepancies in the results of this experiment and in those reported in a previously published paper may be accounted for a number of methodological aspects of the latter report; these may include the use of anesthetic agents in sacrificing of animals, possible contamination of the postmitochondrial and microsomal fractions with cosedimenting fragments of mitochondria, and in insufficient amount of substrate.

0704 RNA POLYMERASE ACTIVITY IN HOMOTRANSPLANTED RAT BRAIN TUMORS INITIALLY INDUCED BY ETHYLNITROSOUREA. (Eng.) Slagel, D. E. (Univ. Kentucky, Coll. Medicine, Lexington, Ky. 40506); Norrell, H. *Acta Neuropathol. (Berl.)* 32(1):1-7; 1975.

Nuclear RNA polymerase activity was studied in homotransplanted rat glial tumors in which the primary tumor was produced by transplacental injection of ethylnitrosourea. Pregnant Sprague-Dawley female

rats were injected iv on the 20th day of gestation. The offspring of these females developed neoplasms of the CNS beginning at six months of age, with peak tumor incidence occurring from 6-8 mo of age. Secondary tumors were produced in 3-6 wk by injecting cultured primary tumor cells into the flank or into the cerebral hemispheres of 1- to 2-day-old Sprague-Dawley rats. Nuclei from secondary tumor cells were tested for RNA polymerase activity in assay solutions, that, in some cases, contained one of the following inhibitors: 250 μg rifampin, 500 μg cycloheximide or 0.02 μg alpha-amanitin. Samples were incubated for 20 min at 37 C, and the activity was measured by liquid scintillation spectrophotometry. Pieces of tumor adjacent to the area used in the assay were examined by light and electron microscopy. The intracranial tumors were basically of two different types: one was a mixed glioma composed of different groups of cells of neuroepithelial origin, and the other was a neurofibrosarcoma containing cells of mesenchymal as well as neuroepithelial origin. The major cytoplasmic feature was the endoplasmic reticulum, which was often packed with fibrous material. The most prominent ultrastructural feature was the large nucleolus, having very prominent nucleolonema with nucleolar associated chromatin. The nuclear RNA polymerase activity was 0.04 nM GTP/mg DNA/20 min in the nuclei of homotransplanted rat flank and intracranial tumors. Alpha-amanitin significantly inhibited RNA polymerase activity in all tumors, indicating that the major nuclear RNA polymerase activity seen *in vitro* in the tumor nuclei was RNA polymerase II. Cycloheximide and rifampin, which have no effect on RNA polymerase activity in normal glial nuclei, inhibited about 20% of the polymerase activity in three of the tumors. The size and multiplicity of the nucleoli in these tumor cells suggests that RNA polymerase I may account for the activity that is inhibited by cycloheximide.

0705 NUCLEAR PROTEIN PATTERNS IN DEVELOPING AND ADULT BRAIN AND IN ETHYLNITROSOUREA-INDUCED NEUROECTODERMAL TUMOURS OF THE RAT. (Eng.) Biessmann, H. (Max Planck Inst. Virus Res., Tübingen, West Germany); Rajewsky, M. F. *J. Neurochem.* 24(2):387-393; 1975.

The patterns of histones and nonhistone proteins in brain chromatin were analyzed in fetal (18th day of gestation), ten-day-old, and adult BD IX rats, as well as in two ethylnitrosourea-induced neuroextodermal tumors (TV1A1 and GV1A1), and in the corresponding malignant cell lines (TV1C1 and GV1C1). Electrophoretic separation of the total nuclear proteins from the various chromatin preparations was performed on 15% polyacrylamide gels containing 2.5 M urea and 1% *N*,*N*'-methylene-bis-acrylamide. Ten percent polyacrylamide gels containing 6.25 M urea and 0.1% *N*,*N*'-methylene-bis-acrylamide were used for further electrophoretic analysis of the nonhistone protein fraction. In spite of an overall similarity, significant quantitative and qualitative differences were found between the nonhistone protein banding patterns of normal brain cell and neoplastic cell chromatin; the nonhistone banding patterns of the cultured malignant cell lines TV1C1 and GV1C1 and those of the corresponding solid tumors TV1A1 and GV1A1 showed no qualitative differences. The nonhistone banding patterns of the normal brain (about 40 different bands) at different stages of development showed both quantitative differences and the presence of particular bands characteristic of fetal or adult brain. Both the fetal and adult nonhistone protein bands also appeared in the electrophoretograms of the neoplastic neuroectodermal cells. These data suggest that the chromatin template activity of neoplastic cells may differ from that of their normal counterpart tissues.

0706 CHEMICAL CARCINOGENESIS IN THE NERVOUS SYSTEM: PREFERENTIAL ACCUMULATION OF O^6-METHYLGUANINE IN RAT BRAIN DEOXYRIBONUCLEIC ACID DURING REPETITIVE ADMINISTRATION OF *N*-METHYL-*N*-NITROSOUREA. (Eng.) Margison, G. P. (Max-Planck-Institut für Hirnforschung, Abteilung Allgemeine Neurologie, 5 Cologne 91, West Germany); Kleihues, P. *Biochem. J.* 148(3):521-525; 1975.

The concentrations of O^6-methylguanine in DNA of various tissues of female BD-IX rats were determined during weekly injections of *N*-[^{3}H]methyl-*N*-nitrosourea (10 mg/kg). Female BD-IX rats were divided into five groups, each consisting of two rats. Group 1 received a single 10 mg/kg injection into the femoral vein and was killed one wk later. The other groups received 2-5 injections at weekly intervals and were killed one wk after the final injection. DNA was isolated by phenol extraction and the DNA hydrolysates were analyzed by chromatography on Sephadex G-10. After five weekly injections of the carcinogen, O^6-methylguanine level in brain DNA (59.70 x 10^{-4} mole% guanine) greatly exceeded that in kidney, spleen and intestine (13.93, 4.86, and 2.79 x 10^{-4} mole% guanine, resp.). In the liver, the final O^6-methylguanine concentration (0.45 x 10^{-4} mole% guanine) was less than 1% of that in brain. Between the first and the fifth injection, the O^6-methylguanine/7-methylguanine ratio in cerebral DNA increased from 0.28 to 0.68. In addition, 3-methylguanine accumulated in brain DNA (8.7 x 10^{-4} mole% guanine) whereas in the other organs no significant quantities were detectable. The kinetics of the increase of O^6-methylguanine in cerebral DNA suggest that there is no major cell fraction in the brain which is capable of excising chemically methylated bases from DNA. This repair deficiency could be a determining factor in the selective induction of nervous system tumors by *N*-methyl-*N*-nitrosourea and other neuro-oncogenic compounds.

0707 EFFECT ON GUINEA-PIGS OF FEEDING NITROSOMORPHOLINE AND ITS PRECURSORS IN COMBINATION WITH ASCORBIC ACID. (Eng.) Akin, F. J. (Agric. Res. Serv., Athens, Ga.); Wasserman, A. E. *Food Cosmet. Toxicol.* 13(2):239-242; 1975.

Nitrosamine toxicity and the nitrosation process in guinea-pigs was investigated. Male and female guinea-pigs of the Duncan Hartly strain were fed ascorbic acid (at a high level of 6.7 g/kg food or a low level of 150 mg/kg food) and/or morpholine

(6.33 g/kg). Nitrosomorpholine (70 mg/1) or $NaNO_2$ (1.0 g/1) was added to the drinking water. Nine groups were used (three males and three females). Each group was fed one of the levels of ascorbic acid or an ascorbic acid-free diet plus one of the treatment regimens. Animals were killed after 25, 9, and 3 weeks of feeding. All combinations of precursors and ascorbic acid or nitrosamine and ascorbic acid were administered. Animals in the three high level ascorbic acid groups remained healthy throughout the 25 weeks. Tissue saturation with ascorbic acid was indicated by presence of ascorbic acid in the urine of these animals during week 24. After two weeks all guinea-pigs receiving no vitamin C in their diets showed classical symptoms of deficiency, and survivors were killed during the third week. Low-level diet animals developed deficiency symptoms after 6-8 weeks and were killed at nine weeks. Precancerous liver lesions were observed in half of the animals receiving nitrosomorpholine. The feeding of ascorbic acid with the nitrosomorpholine did not prevent hepatotoxicity. Full 25-week ingestion of nitrosamine produced most pronounced hepatotoxicity. Moderate liver alterations were seen in the animals ingesting nitrosamine for nine weeks. The feeding of morpholine and $NaNO_2$ together with ascorbic acid did not cause the toxic responses seen with nitrosomorpholine. The authors suggest the possibility that nitrosamine formation was prevented *in vivo* by the ingestion of ascorbic acid with the precursors.

0708 DNA REPAIR SYNTHESIS FOLLOWING EXPOSURE OF GUINEA-PIG PANCREATIC SLICES TO METHYL-N-NITROSOURETHANE *IN VITRO*. (Eng.) Hasumi, K. (Case West. Res. Univ. Sch. Med., Cleveland, Ohio); Iqbal, Z. M.; Alarif, A.; Epstein, S. S. *Experientia* 31(4):467; 1975.

DNA repair synthesis was investigated in male white Hartley guinea pig pancreas slices following *in vitro* exposure to methyl-n-nitrosourethane. The pancreas slices were incubated with 10 mM hydroxyurea for two hr, following which 20 mM methyl-n-nitrosourethane was added to the incubation mixture for 15 min. The slices were washed and incubated for 90 min in fresh medium containing 10 mM hydroxyurea and 10 μCi/ml ^{3}H-methyl-thymidine. Slices were then homogenized; the DNA was isolated; and the DNA content was determined colorimetrically by the diphenylamine reaction. ^{3}H-methyl-thymidine incorporation into DNA in the presence of hydroxyurea was taken as a measure of DNA repair synthesis. Hydroxyurea suppressed the normal DNA replicative synthesis by approximately 77%. However, methyl-n-nitrosourethane treatment resulted in an approximately 7-fold increase over the control in ^{3}H-methyl-thymidine incorporation into DNA in the presence of hydroxyurea. This increase in ^{3}H-methyl-thymidine incorporation into DNA might be attributed to DNA repair synthesis. Previous studies showing that cultured mammalian cells are capable of excising alkylated DNA bases enzymatically suggest the possibility that a similar excision of methyl-n-nitrosourethane-methylated DNA bases followed by DNA repair synthesis occurs in guinea pig pancreas.

0709 ORGAN-SPECIFIC DNA DAMAGE INDUCED IN MICE BY THE ORGANOTROPIC CARCINOGENS 4-NITROQUINOLINE 1-OXIDE AND DIMETHYLNITROSAMINE. (Eng.) Laishes, B. A. (Cancer Res. Cent., Univ. of British Columbia, Vancouver, Canada); Koropatnick, D. J.; Stich, H. F. *Proc. Soc. Biol. Med.* 149(4):978-982; 1975.

The extent of DNA fragmentation induced in lung, kidney, and liver of mice injected with the chemical carcinogens 4-nitroquinoline and dimethylnitrosamine, and with the noncarcinogenic 4-aminoquinoline 1-oxide was estimated by the alkaline sucrose gradient technique, using a new preparation method. This method reduces the usual mechanical shearing of constituent DNA in lung tissue permitting more meaningful comparisons with the results from liver and kidney DNA damage analyses. 4-Nitroquinoline 1-oxide and 4-amino-quinoline 1-oxide in dimethylsulfoxide were administered sc to 4- to 6-mo-old Swiss mice (0.1 ml/25g); dimethylnitrosamine was administered sc in 0.9% sodium chloride (0.1 ml/25g). Following treatment with the carcinogens, the mice were killed and exsanguinated. Lung tissue pieces were minced in 10-15 μl EDTA/saline buffer until all pieces were small enough to pass through the bore of a 10 μl microsampling pipette. The new preparation method involved drawing the fragments into the pipette and layering on an alkaline sucrose gradient overlaid with 0.3 ml of lysing solution. After a 30-min lysis period, another aliquot of lysing solution was added, and the gradients were overlaid with mineral oil and centrifuged. The amount of DNA layered per gradient was estimated from aliquots of liver and kidney cell suspensions, and lung tissue fragments, by the standard diphenylamine technique. The extent of DNA damage induced by 40 mg and 80 mg/4-nitroquinoline occurred in the order: lung, kidney, liver, with no detectable liver DNA damage occurring with 40 mg. The extent of DNA damage induced by dimethylnitrosamine followed the order: liver, kidney, lung. DNA damage was induced by 4-aminoquinoline 1-oxide. The sites of greatest DNA damage appeared to correlate with sites of high levels of DNA repair synthesis and the sites of tumor induction; this suggests that a DNA fragmentation and DNA repair synthesis occur in the target tissues from which tumors arise following the application of organotropic carcinogens.

0710 ANALYSES OF DIFFERENTIAL SENSITIVITIES OF SYNCHRONIZED HeLa S3 CELLS TO RADIATIONS AND CHEMICAL CARCINOGENS DURING THE CELL CYCLE. III. 4-NITROQUINOLINE I-OXIDE AND ITS DERIVATIVES. (Eng.) Watanabe, M. (Fac. Pharm. Sci., Kanazawa Univ., Kanazawa 920, Japan); Horikawa, M. *Mutat. Res.* 28 (2):295-304; 1975.

The sensitivity of synchronized HeLa S3 cells to the chemical carcinogens 4-nitroquinoline 1-oxide (4-NQO) and its derivatives was measured, and chemical bindings of these carcinogens with cellular DNA and rates of their excision in different phases of the cell cycle were determined. The cyclic variation curves of sensitivity to 4-NQO and 4-hydroxyaminoquinoline 1-oxide (4-HAQO) through the cell cycle of HeLa S3 cells, determined as the loss of the colony-forming

ability of cells treated with 5 x 10^{-9} M 4-NQO and 1 x 10^{-5} M 4-HAQO for 20 min, showed almost the same tendency. The sensitivity to 4-NQO and 4-HAQO through the cell cycle decreased from the late S to early G2 phases. Cells in other phases were relatively sensitive to both carcinogens. The radioactivity of [^{3}H] 4-HQO and [^{3}H]4-HAQO in DNA of the synchronous HeLa S3 cells was measured immediately after treatment with 2.5 x 10^{-5} M[^{3}H]4-NQO and [^{3}H]4-HAQO during different phases of the cell cycle. The amounts of 4-NQO and 4-HAQO bound with cellular DNA during the cell cycle seem to be closely related to the cyclic variation curves of the sensitivity to these chemical carcinogens. 4-HQO and 4-HAQO were bound specifically with cellular DNA in the mitotic phase to the middle of the S phase, phases highly sensitive to these carcinogens; very little binding occurred in late S phase, which was rather insensitive to these carcinogens. There was no significant difference in excision rates of these carcinogens from the DNA of HeLa S3 cells through the cell cycle. These findings indicate that the cyclic variation of 4-NQO and 4-NAQO cell survival during the cell cycle may be due to differences in the amounts of 4-NQO and 4-HAQO bound with cellular DNA.

0711 THE MAJOR CAUSE OF INACTIVATION AND MUTATION BY 4-NITROQUINOLINE 1-OXIDE IN *ESCHERICHIA COLI:* EXCISABLE 4NQO-PURINE ADDUCTS. (Eng.) Ikenaga, M. (Fac. Med., Osaka Univ., Japan); Ichikawa-Ryo, H.; Kondo, S. *J. Mol. Biol.* 92(2): 341-356; 1975.

A study was undertaken to determine the major cause of inactivation and mutation by 4-nitroquinoline 1-oxide (4NQO) in *Escherichia coli*. Strains used were *E. coli* B strain, H/r30, and its *uvrA*⁻ derivative Hs30R. DNA was extracted from cells treated with [^{3}H]4NQO and subjected to radiochromatography. Two peaks of 4-NQO-guanine adduct, one peak of 4NQO-adenine adduct, and a peak due to 4-aminoquinoline 1-oxide released from a labile fraction of 4NQO-guanine adducts during acid hydrolysis of DNA were detected. The four types of adducts disappeared from DNA of the normal strain at almost the same rate (about 85% on 60 min by postincubating in nutrient broth), but did not disappear from the *uvrA*⁻ derivative lacking the excision-repair ability for UV-induced pyrimidine dimers; there was, however, a slight disappearance of 4-aminoquinoline 1-oxide-releasing adduct. The number of DNA lesions/genome of *uvrA*⁻ strain at 37% survival was found to be nearly equal between the 4NQO-purine adducts (~200 lesions) and pyrimidine dimers (~100 lesions). At the molecular level, these findings quantitatively paralleled previous findings at the cellular level that *uvrA*⁻ strain was about 25-30 times as sensitive as its parental strain to killing and mutation by either 4NQO or UV light. The authors conclude that 4NQO effects on *E. coli* are primarily due to covalent binding of 4NQO to DNA producing 4NQO-guanine and 4NQO-adenine adducts in a ratio of about 4:1 without repair and about 7:1 with repair. Thus, *uvrA* and *uvrB* genes control the first step in excision repair capable of repairing various DNA lesions other than pyrimidine dimers.

0712 GENETIC CHARACTERIZATION OF ADENINE-3 MUTANTS INDUCED BY 4-NITROQUINOLINE 1-OXIDE AND 4-HYDROXYAMINOQUINOLINE 1-OXIDE IN *NEUROSPORA CRASSA*. (Eng.) Ong, T. (Natl. Inst. Environ. Health Sci., Res. Triangle Park, N. C.); Matter, B. E.; de Serres, F. J. *Cancer Res.* 35(2):291-295; 1975.

Specific locus mutations induced by the chemical carcinogens, 4-nitroquinoline 1-oxide (4NQO) and 4-hydroxyaminoquinoline 1-oxide (4HAQO) were characterized to obtain a presumptive identification of the genetic alterations at the molecular level. One hundred and eighty-four 4NQO-induced and 219 4HAQO-induced *ad-3* mutants of *Neurospora crassa* were studied with a series of genetic tests to determine their genotype and the frequencies of point mutations and multilocus deletions. The spectrum of *ad-3* mutants among 4NQO-induced mutants was similar to that of 4HAQO-induced mutants. None of the mutants was a multilocus deletion mutant. The ratio of *ad-3A* genotype (33%) to *ad-3B* genotype (67%) mutants was the same in the two samples, as were the frequencies of complementing *ad-3B* mutants (63% of the 4NQO-induced mutants and 68% of the 4HAQO-induced mutants) suggesting that the mechanism of mutation induction by 4NQO in *Neurospora crassa* is identical to that of 4HAQO. It is not clear, however, whether 4NQO is mutagenic itself or whether reduction of 4NQO to 4-hydroxyaminoquinoline is the first step involved in the mutagenicity of this compound. Heterokaryon tests indicated that the relatively high frequencies of 4NQO- or 4HAQO-induced *ad-3B* mutants show allelic complementation and that most of the complementing *ad-3B* mutants (74% 4NQO-induced and 71% 4HAQO-induced) have nonpolarized complementation patterns. It is concluded that both agents induce predominantly base-paired substitution mutations.

0713 PANCREATIC NEOPLASMS IN AN ANIMAL MODEL: MORPHOLOGICAL, BIOLOGICAL, AND COMPARATIVE STUDIES. (Eng.) Pour, P. (Univ. Nebraska Medical Center, 42nd and Dewey Ave., Omaha, Nebr. 68105); Mohr, U.; Cardesa, A.; Althoff, J.; Kruger, F. W. *Cancer* 36(2):379-389; 1975.

Syrian golden hamsters received 2,2'-dihydroxy-di-*n*-propylnitrosamine in an attempt to induce pancreatic neoplasms similar to those in man. Three groups of 20 males and 20 females each received weekly sc injections for life at doses of 500/mg/kg, 250 mg/kg, or 125 mg/kg. Following complete autopsies (the last treated animal died in the 44th wk), tissues were examined histologically. Treated animals developed diarrhea and presented weight losses at about 16 wk after beginning the treatment. The weight loss was most pronounced in the high-dose group and more intense in males than in females. The overall incidence of pancreatic neoplasms was 100%. Gross examination indicated that most neoplasms were in the head of the pancreas and less frequently in its body. Histologic examination, however, revealed that only 6% of the tumors were actually confined to the head; in most cases the multiple neoplasms were distributed throughout the pancreas and were either adenomas or carcinomas. Intraductal carcinomas appeared as early as 15 wk. Most carcinomas were well-differentiated, presenting glandular, cystic, papillary, or cystic-

papillary formations. Less differentiated varieties of adenocarcinomas, showing tubular or scirrhous patterns and solid strands or sarcoma-like structures, also occurred. An invasion of the blood vessels, lymph nodes, perineural lymphatics, peritoneum, spleen and stomach was found in 18 hamsters. Distant metastases occurred in the liver of five males and two females and in the lungs of two males and one female. Necrosis, calcification, and giant cell inflammation of the peritoneum (two males), the perirenal tissues (one male), and the lungs (two males, two females), were found in hamsters with invading acinar cell carcinomas. Vascular thrombosis was seen in four cases, occurring in the lungs, splenic vein, portal vein, and renal artery. These findings are all comparable with previously published observations on pancreatic cancer in man; it is thus concluded that the pancreatic neoplasms induced in the Syrian hamster resemble those of man, both morphologically and biologically.

0714 CARCINOGENICITY OF 5-NITROFURANS AND RELATED COMPOUNDS WITH AMINO-HETERO-CYCLIC SUBSTITUENTS. (Eng.) Cohen, S. M. (St. Vincent Hosp., Worcester, Mass.); Ertürk, E.; Von Esch, A. M.; Crovetti, A. J.; Bryan, G. T. *J. Natl. Cancer Inst.* 54(4):841-850; 1975.

Carcinogenicity of eight 5-nitrofurans with heterocyclic substitutents at the 2-position of the furan ring was investigated by feeding the chemicals to weanling female Sprague-Dawley rats. The chemicals were fed at doses ranging from 0.010-0.200% of the diet for up to 75 wk. The rats were weighed, and food and chemical consumption was determined at wk 0, 1, 3, 6, and 10, and monthly thereafter. Rats were palpated for tumors twice weekly, beginning at the sixth wk. *N*-[5-(5-nitro-2-furyl)-1,3,4-thiadiazol-2-yl]acetamide induced in 30 rats the highest incidence of tumors with the greatest number of tissues involved: forestomach squamous cell tumors (22), kidney pelvis transitional cell carcinomas (15), pulmonary alveolar cell carcinomas (16), hemangioendothelialsarcomas (20) of the intestine, mesentery, liver, lung, and pancreas, and a few tumors of other tissues. 2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole, 2-amino-5-(5-nitro-2-furyl)-1,3,4-oxadiazole, and *trans*-2-[(dimethylamino) methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole produced high incidences of mammary tumors. The other four 5-nitrofurans tested: *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide; 2,2,2-trifluoro-*N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide; 5-(5-nitro-2-furyl)-1,3,4-oxadiazol-2-ol; and *N*-{[3-(5-nitro-2-furyl)-1,2,4-oxadiazol-5-yl]methyl} acetamide were associated with tumor incidences of 40-60%. Two other chemicals were also tested: 2-amino-5-nitrothiazole caused a low incidence of breast and kidney pelvic tumors and 2-amino-4-(*p*-nitrophenyl)thiazole induced a high incidence of breast and salivary gland adenocarcinomas and lymphomas. Two possibilities are suggested for the carcinogenicity of these substituted 5-nitrofurans: 1) the molecule could be activated at both ends (the 5-nitro group and the substituent at the 2-position of the furan ring), and thus react with macromolecules with either substituent or both. The requirement of two reactive centers could explain the lack of carcinogenicity of some 5-nitrofurans and of analogs of nitrofurans without the nitro group, or 2) the substituent at the 2-position of the furan ring could modify the chemical properties of the 5-nitrofurans and result in variations in the biologic properties of the different 5-nitrofurans. The eventual interaction of the nitro group with cellular target molecules would cause the transformation of a normal cell to a cancer cell.

0715 TRANSLOCATION KINETICS OF PLUTONIUM OXIDE FROM THE POPLITEAL LYMPH NODES OF BEAGLES. (Eng.) Dagle, G. E. (Biology Dept., Battelle Pacific Northwest Lab., Richland, Wash. 99352); Lebel, J. L.; Phemister, R. D.; Watters, R. L.; Gomez, L. S. *Health Phys.* 28(4):395-398; 1975.

The translocation kinetics of high-fired plutonium oxide from the popliteal lymph nodes was studied in beagles with 9.2-39.4 μCi of plutonium-239 oxide implanted s.c. in the left hind paws to simulate accidental contamination of hand wounds. The left hind paw was amputated four wk after implantation to prevent continued deposition of plutonium oxide particles in the left popliteal lymph node. The injected paws and corresponding popliteal lymph nodes were monitored *in situ* approximately twice a week for radioactivity with a NaI wound counter. The counting data showed no clearance of plutonium from the popliteal lymph nodes from dogs sacrificed eight wk after plutonium injection and slight clearance of plutonium from the popliteal lymph nodes of beagles monitored for 32 wk. The estimated time for the fraction of plutonium retained at 100 days to be reduced by 1/2 varied from 290 to 1508 days. Treatment with penthanil (0.25 g, i.v.) did not have a measurable effect on the clearance of high-fired plutonium oxide from popliteal lymph nodes. The results show that popliteal lymph nodes are major deposition sites of plutonium oxide injected into the hind paws of beagles and are consistent with the known importance of the superficial cervical lymph nodes in the translocation of plutonium from the front paws of beagles, and the tracheobronchial lymph nodes in the translocation of plutonium from the lungs of beagles.

0716 PURINE AND PYRIMIDINE MODIFICATION OF GROWTH AND SURFACE PROPERTIES OF BABY HAMSTER KIDNEY CELLS (BHK21/C13). (Eng.) Taylor, J. C. (Univ. Utah Coll. Med., Salt Lake City); Hill, D. W.; Rogolsky, M. *Exp. Cell Res.* 90(2):468-471; 1975.

BHK21/C13 hamster kidney cells were grown in culture medium supplemented with thymidine (Thy) and hypoxanthine (Hpx) or adenosine (Ad) to study: growth characteristics, Concanavalin A (Con A) agglutinability and ability to synthesize plasminogen activator (PA). Cells were grown in Eagles minimal essential medium with 10% calf serum, non-essential amino acids, penicillin and streptomycin. High passage cells were grown in the same medium but with 5% calf

serum. Cells were suspended in agar and grown for ten days at 37 C in 5% CO_2. The agar was then overlayed with casein-fibrin solution, incubated at 37 C overnight and observed for liquefaction of fibrin and/or hydrolysis of casein. Agglutination with Con A was tested after cells were removed from culture in a 0.02% disodium versenate solution, centrifuged and resuspended in a calcium- and magnesium-deficient saline. Con A was added, the solution was incubated at room temperature for 20 min and the cells were examined microscopically for agglutination. BHK21/C13 cells did not grow in agar or produce PA, and they were not agglutinable by Con A. High passage cells demonstrated PA activity and 50% were agglutinated by 10 μg/ml of Con A. Cells treated with Hpx (7.14×10^{-5} M) and Thy (3.12×10^{-5} M) demonstrated colonization in agar and PA activity, as did cells treated with Ad (1.59×10^{-4} M) and Thy (1.46×10^{-5} M). 50% of Hpx- and Thy-treated cells were agglutinated by 42 μg/ml Con A, while the maximum number of cells treated with Ad and Thy were agglutinated by 84 μg/ml Con A. It was found that the characteristics of high passage cells could be transferred to BHK21/C13 cells by DNA from the high passage strains. It is suggested that PA activity and Con A agglutinability correlate positively and that these characteristics may be related to growth in agar.

0717 SULFHYDRYL DEPENDENCE IN PRIMARY EXPLANT HEMATOPOIETIC CELLS. INHIBITION OF GROWTH *IN VITRO* WITH VITAMIN B_{12} COMPOUNDS. (Eng.) Toohey, J. I. (Dep. Med., Univ. California, Los Angeles). *Proc. Natl. Acad. Sci. USA* 72(1):73-77; 1975.

Sulfhydryl dependence and the inhibitory effect of vitamin B_{12} compounds were investigated in primary explant cells of three murine leukemias and of normal mouse bone marrow. Sulfhydryl compounds used in the study include dithiothreitol, mercaptoethanol, thioglycerol, thioethanolamine, cysteine, reduced glutathione, oxidized glutathione, co-enzyme A, and sodium thioglycolate. Primary explants of P388, EL-4, and L1210 cells and the mouse bone marrow all required sulfhydryl compounds for proliferation or colony formation *in vitro* to varying degrees. P388 and L1210 showed an initial absolute dependence on sulfhydryl compounds; the cells died within three days in the absence of sulfhydryl supplementation. EL-4 leukemia cells, although stimulated to undergo immediate proliferation by addition of sulfhydryl compounds, could proliferate *in vitro* in the absence of sulfhydryl compounds after a lag period of several days. Mouse bone marrow colony formation occurred in basal medium but was markedly stimulated by sulfhydryl supplementation and inhibited by sulfhydryl depletion. Nine established cell lines (mouse fibroblasts, rat XC sarcoma, rat Walker-256 carcinosarcoma, human monocytic leukemia-Jill, human lymphoblastoid cells of T type-Molt 4 and of B type-LA 96, Gross-virus-infected mouse lymphoblastoid cells, mouse lymphoblastic leukemia-L1210 cell line, and Friend-virus-infected mouse leukemia cell line) showed no stimulation by these compounds. The various sulfhydryl compounds had widely differing potencies in promoting *in vitro* proliferation of dependent cells. The effect appeared to be specific for sulfhydryl groups in the reduced form. Vitamin B_{12} compounds inhibited the growth of sulfhydryl-requiring cells, apparently by catalyzing the oxidation of the sulfhydryl groups. These data indicate that certain malignant cells have a requirement for sulfhydryl compounds when first explanted from the animal, suggesting that the requirement exists *in vivo* and is fulfilled by natural sulfhydryl compounds in the body. The ability of vitamin B_{12} compounds to prevent proliferation of the freshly explanted malignant cells leads to the speculation that the more active B_{12} compounds might be capable of inhibiting proliferation of sulfhydryl-dependent cells *in vivo*.

0718 RAUWOLFIA DERIVATIVES AND BREAST CANCER. A CASE CONTROL STUDY IN OLMSTEAD COUNTY, MINNESOTA. (Eng.) O'Fallon, W. M. (Mayo Clinic and Mayo Foundation, Rochester, Minn.); Labarthe, D. R.; Kurland, L. T. *Lancet* 2(7929):292-296; 1975.

In response to three reports of an association between rauwolfia derivatives (reserpine, rescinnamine and methoserpidine) and breast cancer, a case/control study was undertaken comparing 450 women with breast cancer to an age-matched group of 475 women with cholelithiasis. All available clinical records were reviewed for demographic, epidemiologic, and clinical data deemed necessary to the study. A user of any antihypertensive drug (rauwolfia or other) was defined as anyone whose record indicated a prescription for any concentration of the drug at any time more than six months before the defining diagnosis. Cases and controls were compared with respect to a number of characteristics including age at diagnosis, date of diagnosis, marital status, education, gravidity, parity, age at first pregnancy, height, weight, and the Quetelet index of body mass. There were no meaningful differences in the two groups, except with respect to a history of hypertension; 167 of 450 breast cancer cases (37%) had hypertension as compared with 225 of 475 controls (47%). However, the rates of use of rauwolfia derivatives by hypertensive breast cancer cases and controls were identical; this indicates that an association between the use of rauwolfia derivatives and breast cancer is unlikely.

0719 A THEORETICAL STUDY OF TRICYCLOQUINAZOLINE CARCINOGENESIS. (Eng.) Hall, G. G. (Dept. Mathematics, Univ. Nottingham, NG7, 2RD, England); Rodwell, W. R. *J. Theor. Biol.* 50(1):107-120; 1975.

Relationships between electronic structures and carcinogenic activities were investigated in a series of molecules of which the parent is tricycloquinazoline. The carcinogens among these molecules do not belong to any of the better known classes of carcinogens and small variations between the members of the series produced marked differences in carcinogenic activity. Using semi-empirical methods of pi-molecular orbital therory, calculations were made on the electronic structures of a number of the tricycloquinazoline series and the effect of substitutents on the charge distribution was examined through the cal-

culation of atom-atom polarizabilities. The relation of these results to the carcinogenic behavior of the tricycloquinazolines and the form of the variation of carcinogenic activity among the series of molecules studied was examined critically, and the hypothesis that the specificity of tricycloquinazoline carcinogenesis could be due to electrostatic interactions between DNA base pairs and tricycloquinazoline molecules intercalated into the DNA helix was formulated. Theoretical calculations to test this hypothesis are presented. Although the magnitudes of the electrostatic interactions were large enough to be significant, no general correlation in support of the hypothesis was found. Ionization potentials of tricycloquinazolines were also calculated and these support the view that there is no correlation between ionization potential and carcinogenic activity.

0720 EFFECT OF PRENATAL ADMINISTRATION OF T-2 TOXIN TO MICE. (Eng.) Stanford, G. K. (Dep. Biol., Univ. Alabama, University); Hood, R. D.; Hayes, A. W. *Res. Commun. Chem. Pathol. Pharmacol.* 10(4):743-746; 1975.

The effect of T-2 toxin on mammalian embryos was demonstrated. T-2 toxin is a member of the sesquiterpene toxins produced by *Fusarium tricinctum* at low temperatures. Pregnant female mice were injected i.p. with T-2 toxin (0.5, 1.0 or 1.5 mg/kg) dissolved in propylene glycol (0.1 ml) on one of days 7-11 of gestation. Controls received solvent or no treatment. Uterine horns were examined for resorption sites on day 18. Dosages of 1.0 or 1.5 mg/kg produced a number of maternal deaths within two days. Administration of 0.5 mg/kg did not significantly affect fetal survival except on day 11 of gestation. On day 10 approximately 37% of fetuses from females given 1.0 or 1.5 mg/kg T-2 toxin were grossly malformed. Anomalies were bent, shortened or missing tails; limb malformations, including oligodactyly and syndactyly; exencephaly; open eye; and retarded jaws. In the untreated controls there was one malformation (exencephaly). On days 9 and 10, 17 and 42%, resp. of fetuses showed at least one skeletal defect. Fetuses treated with 0.5 mg/kg on days 9 and 10 had skeletal abnormalities of 4% and 10%, resp. Skeletal anomalies consisted of fused or malformed vertebrae and fused ribs. Fetuses treated on gestation days 7, 8 or 11 did not have these defects. The fetuses from females exposed to toxin (particularly on gestation days 7, 10 and 11) showed a decrease in weight when compared to fetuses from solvent-treated animals. The same developmental stages were most susceptible to T-2 toxin's lethal effect and its growth depressant effect. Further study of fungal toxins in regard to their effect on development and embryotoxicity is suggested.

0721 DIETARY VITAMIN A AND HUMAN LUNG CANCER. (Eng.) Bjelke, E. (Cancer Regist. Norway, Montebello). *Int. J. Cancer* 15(4):561-565; 1975.

Five-yr follow-up results are reported for 8,278 Norwegian men, who in mail surveys had reported their cigarette smoking and dietary habits. The men considered in this survey had reported their cigarette smoking habits in 1964, had returned dietary information sufficiently complete for vitamin A intake to be estimated in 1967, and were alive in January 1968. Thirty-six cases of cancer of the bronchus or lung were found, all first diagnosed between 1968 and 1972. The diagnosis was supported by histologic examination in 25 cases. A lower lung cancer rate in those with the high values of vitamin A intake was seen in all age groups, and in both urban and rural residents. Neither the total group of lung cancers, nor the subset of proven carcinomas other than adenocarcinoma, showed notable age differences in regard to their associations with the vitamin A index. A positive relationship between cigarette smoking and lung cancer appeared at both high and low levels of vitamin A intake. None of the dietary variables that notably correlated with vitamin A intake showed a similarly strong negative association with lung cancer. The findings suggest that vitamin A active compounds or some closely associated dietary factors may modify the expression of pulmonary carcinogens or cocarcinogens in man.

0722 PRIMARY HEPATIC CARCINOGENESIS IN RATS UNDER THE INFLUENCE OF A PESTICIDE, 2-CHLOROETHYL 1-METHYL-2-(*p*-tert-BUTYLPHENOXY)ETHYL SULFITE (ARAMITE). (Fre.) Truhaut, R. (Faculte des Sciences Pharmaceutiques et Biologiques, Universite Rene-Descartes, Paris-V, 4, avenue de l'Observatoire, 75006 Paris, France); Claude, J.-R.; Vu Ngoc Huyen; Warnet, J.-M.; Blanc, F. *C. R. Acad. Sci. [D] (Paris).* 281(9):599-604; 1975.

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0760 INDUCTION OF MALIGNANT PANCREATIC TUMORS BY LOCAL APPLICATION OF 7, 12-DIMETHYLBENZANTHRACENE [abstract]. (Eng.) Mills, L. R. (V.A. Hospital and Medical Coll. Georgia, Augusta); Dissin, J.; Mainz, D. L.; Black, O.; Webster, P. D. *Fed. Proc.* 34(3):827; 1975.

0761 MOLECULAR STRUCTURES OF SOME CARCINOGENS RELATED TO 7,12-DIMETHYL BENZ[a]ANTHRACENE [abstract]. (Eng.) Glusker, J. P. (Inst. Cancer Res., Philadelphia, Pa.); Carrell, H. L.; Zacharias, D. E. *Fed. Proc.* 34(3):663; 1975.

0762 EFFECTS OF 7,12 DIMETHYLBENZ(α)ANTHRACENE ON CELL PROLIFERATION KINETICS OF HAMSTER CHEEK POUCH EPITHELIUM [abstract]. (Eng.) Izquierdo, J. N. (Univ. Rochester, N.Y.) *Diss. Abstr. Int. B* 35(7):3419; 1975.

0763 SUSPECTED PRENEOPLASTIC NATURE OF MAMMARY NODULES (HAN) DERIVED FROM ORGAN CULTURE [abstract]. (Eng.) Lin, F. K. (Tumor Biol. Lab., Univ. Nebraska, Lincoln); Banerjee, M. R. *Proc. Am. Assoc. Cancer Res.* 16:91; 1975.

0764 THE SIGNIFICANCE OF POLYCYCLIC HYDROCARBON PRETREATMENT ON THE BIOAVAILABILITY OF PHENACETIN (P) IN THE RAT [abstract]. (Eng.) DeAngelis, R. L. (Wellcome Res. Lab., Research Triangle Park, N.C.); Welch, R. M. *Fed. Proc.* 34(3): 742; 1975.

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0771 HIGHLY PURIFIED LIVER MICROSOMAL CYTOCHROME P-448 FROM 3-METHYLCHOLANTHRENE (3-MC) TREATED RATS [abstract]. (Eng.) Ryan, D. (Dep. Biochem. Drug Metab., Hoffmann-La Roche Inc., Nutley, N.J.); Lu, A. Y. H.; West, S.; Lai, C. Y.; Chang, D.; Levin, W. *Fed. Proc.* 34(3):729; 1975.

0772 EFFECT OF PHENOBARBITAL (PB) AND 3-METHYLCHOLANTHRENE (3MC) ADMINISTRATION ON HEPATIC EPOXIDE HYDRASE(S) LEVELS IN RAT LIVER MICROSOMES [abstract]. (Eng.) Mukhtar, H. (Med. Coll. Georgia, Augusta); Jerina, D. M.; Bresnick, E. *Fed. Proc.* 34(3):726; 1975.

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0777 DETECTION OF ARYLHYDROXYLAMINES AS INTERMEDIATES IN THE METABOLIC REDUCTION OF NITRO COMPOUNDS. (Eng.) Sternson, L. A. (Sch. Pharm., Univ. Georgia, Athens). *Experientia* 31(3): 268-270; 1975.

0778 PHARMACOKINETICS OF 1-(2-CHLOROETHYL)-3-CYCLOHEXYL-1-NITROSOUREA (CCNU) IN THE RAT [abstract]. Reed, D. J. (Oregon State Univ., Corvallis). *Proc. Am. Assoc. Cancer Res.* 16: 92; 1975.

0779 A RESPONSE OF PLANT SEEDLINGS TO THE EFFECT OF N-NITROSO-N-METHYLUREA. (Rus.) Slepian, E. I. (V. L. Komarov Inst. Botany U.S.S.R. Acad. Sciences, Leningrad, U.S.S.R.): Gabaraeva, N. I. *Vopr. Onkol.* 21(5): 94-98; 1975.

0780 DIFFERENTIAL EFFECT OF PHENOBARBITONE, PREGNENOLONE-16α-CARBONITRILE AND AMINO-ACETONITRILE ON DIALKYLNITROSAMINE METABOLISM AND MUTAGENICITY *IN VITRO*. (Eng.) Bartsch, H. (Int. Agency Res. Cancer, Lyon, France); Malaveille, C.; Montesano, R. *Chem. Biol. Interact.* 10(5):377-382; 1975.

0781 ON THE REACTIONS OF $NaNO_2$ WITH ETHYL UREA IN THE PRESENCE AND ABSENCE OF ASCORBIC ACID. (Eng.) Synnott, J. A. (Dep. Chem., Univ. New Brunswick, Fredericton, Canada); Unger, I.; Strunz, G. *Naturwissenschaften* 62(3):138-139; 1975.

0782 USE OF MUTAGENIC AGENTS IN IMPROVEMENT OF α-AMYLASE PRODUCTION BY *BACILLUS SUBTILIS*. (Eng.) Bailey, M. J. (Biotech. Lab., Technical Res. Center Finland, Helsinki); Markkanen, P. H. *J. Appl. Chem. Biotechnol.* 25(1):73-79; 1975.

0783 FORMATION OF N-NITROSOPYRROLIDINE FROM PROLINE AND COLLAGEN. (Eng.) Gray, J. I. (Dep. Food Sci. Hum. Nutr., Michigan State Univ., E. Lansing); Dugan, L. R., Jr. *J. Food Sci.* 40(3): 484-487; 1975.

0784 COMPARATIVE STUDIES CONCERNING THE SUITABILITY OF EUROPEAN HAMSTERS AND SYRIAN GOLDEN HAMSTERS FOR INVESTIGATIONS ON SMOKE EXPOSURE. (Eng.) Reznik, G. (Medizinische Hochschule Hannover, 3000 Hannover-Kleefeld, Karl-Wiechert-Allee 9, West Germany); Reznik-Schüller, H.; Schostek, H.; Deppe, K.; Mohr*, U. *Arzneim. Forsch.* 25(6):923-926; 1975.

See also:

* (Rev): 601, 602, 603, 604, 612, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630
* (Phys): 795, 796
* (Viral): 836, 886
* (Immun): 890, 904, 908, 964, 965, 967, 973, 974
* (Path): 1002, 1003, 1020, 1037, 1056, 1059, 1099, 1101
* (Epid-Biom): 1106, 1107, 1108, 1112, 1121, 1123

0785 THE RELATIONSHIP BETWEEN THE LATENT PERIOD AND ANIMAL AGE IN THE DEVELOPMENT OF FOREIGN BODY SARCOMAS. (Eng.) Paulini, K. (Cent. Biol. Theor. Med., Univ. Ulm, West Germany); Beneke, G.; Körner, B.; Enders, R. *Beitr. Pathol.* 154(2): 161-169; 1975.

The influence of animal age and the latent period of sarcogenesis in the development of foreign body sarcoma was studied in a population of 118 male SIV-50 rats, aged 1-15½ months. Polyester-polyurethane sponges with a density of 35 kg/m^3 were implanted paravertebrally in the subcutaneous connective tissue; 99 animals received implants of four cylindrical sponges 12 mm in diameter and six mm thick, while 19 animals were implanted with two rectangular sponges (120 x 20 x 6 mm). Animals receiving cylindrical implants showed a tumor formation rate of 12.1%, while those with rectangular implants had a rate of 23.6%. Tumors occurred in animals of considerable age (20.6 months average), regardless of the age at implantation. The approximately 13.4-month latent period showed an inverse proportion to the age of animals at implantation. The development of tumors in young animals required a longer latent period than that for older rats. Thus, the age of the animal, and not the latent period, is believed to be the significant factor in the development of foreign body sarcomas. These results also confirm previously published reports showing that the shape and size of the implant plays an important role in sarcogenesis after foreign body implantation.

0786 FOREIGN-BODY TUMORIGENESIS: NUMBER, DISTRIBUTION, AND CELL DENSITY OF PRENEOPLASTIC CLONES. (Eng.) Thomassen, M. J. (Univ. Hosp. Cleveland, Ohio); Buoen, L. C.; Brand, K. G. *J. Natl. Cancer Inst.* 54(1):203-207; 1975.

The number of preneoplastic clones was calculated in foreign body tumorigenesis and their distribution patterns on foreign body surfaces were analyzed in an investigation which was undertaken primarily to determine how many different preneoplastic parent cells are mobilized in an animal responding to foreign body implantation. Double 15 x 22 mm plastic films (rigid and unplasticized vinyl chloride acetate copolymer; two films on top of each other) were implanted s.c. in CBA/H-T6 mice. After 7.5 (group 1), 8.5 (group 2), and 9.5 (group 3) months, the films were removed and each interior film covered by a cell monolayer on one side only was cut in four 7 x 10 mm segments. The film segments were then transferred to (CBA/H x CBA/Br)F_1 or (CBA/H-T6 x CBA/Br)F_1 recipient mice which carried preformed foreign body-reactive tissue capsules, induced two months earlier by implantation of glass coverslips. Each segment was placed in the preformed tissue capsule with the cell area pointing toward the body wall of the animal. Tumors arising from segments of the same implant and from the corresponding original capsule were examined for possible homology by: *in vitro* culture and chromosomal analysis; preparation of histologic sections stained with hematoxylin and eosin for determination of the histopathologic sarcoma type and the grade of anaplasticity; tumor growth characteristics and growth rate *in vivo* on transplantation of 1-mm^3 tumor specimens to histocompatible recipients and *in vitro;* and evaluation of tumor latencies. Definite homology was established in 2/9 tumor-positive donor animals in group 1 and in 2/9 tumor-positive animals in group 2. Five tumors of definite homology were seen among 11 positive donors in group 3. It is concluded that preneoplastic clones were present in limited numbers and that they were either widely disseminated or spatially restricted on the implant surfaces. It is further concluded that for clone dissemination, the clonal cells must reach a crucial stage of preneoplastic maturity and/or the foreign body-reactive capsule must be in a condition permitting cells to complete preneoplastic maturation and achieve neoplastic autonomy.

0787 ETIOLOGICAL FACTORS, STAGES, AND THE ROLE OF THE FOREIGN BODY IN FOREIGN BODY TUMORIGENESIS: A REVIEW. (Eng.) Brand, K. G. (Med. Sch., Univ. Minnesota, Minneapolis); Buoen, L. C.; Johnson, K. H.; Brand, I. *Cancer Res.* 35(2):279-286; 1975.

Attempts were made to correlate previously published data and observations on the process of foreign body tumorigenesis with the course of the tumorigenic foreign body reaction as studied histologically and by electron microscopy. Plastic films (unplasticized vinyl chloride acetate copolymer, 15 x 22 x 0.2 mm) were implanted in CBA/H or CBA/H-T6 mice. After various time periods, the implants were excised, cut into smaller segments, and transferred separately into recipient mice which were fully histocompatible, yet distinguishable from the donor mouse. Sarcomas of donor origin developed in the recipients up to two yr later. Tumors that arose from segments of the same original implant were often homologous. Similar experiments were conducted to determine appearance time and location of preneoplastic cells relative to the course of foreign body reaction. The earliest successful demonstration of preneoplastic cells on the foreign body surface was accomplished at 11 weeks postimplantation. From these observations it seems that the process of foreign body tumorigenesis proceeds through several stages, each seemingly providing specific conditions required sequentially for preneoplastic maturation as expressed by the following criteria: cellular proliferation and tissue infiltration during acute foreign body reaction; fibrosis of the tissue capsule surrounding the foreign body; quiescence of the tissue reaction; and availability of a foreign body surface for direct contact with clonal preneoplastic cells. There is no indication that the initial acquisition of neoplastic potential and the determination of specific tumor characteristics are based on direct physical or chemical reactions between cells and the foreign body. These key etiological events presumably occur in mesenchymal stem cells associated with the microvasculature no later than during the acute stage of foreign body reaction and long before clonal descendants of these cells are first found in contact with the foreign body surface. It is suggested that cells with neoplastic determina-

tion may be present in normal tissue prior to the introduction of a foreign body and that the foreign body only creates the conditions required for stepwise preneoplastic maturation.

0788 EFFECTS OF γ-IRRADIATION ON BIOSYNTHESIS OF DIFFERENT TYPES OF RIBONUCLEIC ACIDS IN NORMAL AND REGENERATING RAT LIVER. (Eng.) Markov, G. G. (Bulgarian Acad. Sci., Sofia); Dessev, G. N.; Russev, G. C.; Tsanev, R. G. *Biochem. J.* 146(1): 41-51; 1975.

The hypothesis that irradiation temporarily stimulates the transcription of messenger RNA on genes operating at the time of irradiation, but suppresses the synthesis of new types of messenger RNA by affecting the mechanisms of activation of new genes, was studied. To this end, the effect of λ-irradiation (4000 rads) on the synthesis of ribosomal and heterogeneous nuclear RNA (pre-messenger RNA) was tested. Normal and regenerating rat liver was studied using 40 min labeling with [6-^{14}C]orotic acid. Liver regeneration was induced by removing 2/3 of the liver. Irradiation did not change the specific radioactivity of the endogenous RNA precursors in intact animals, but partial hepatectomy strongly affected the endogenous pool. Irradiation enhanced the synthesis of preribosomal RNA for at least 12 hr. The synthesis of pre-messenger RNA was enhanced for the first three hr after irradiation. Partial hepatectomy strongly stimulated the synthesis of both preribosomal RNA and pre-messenger RNA. Pre-ribosomal RNA synthesis was enhanced in regenerating liver of animals irradiated before or after the operation, leading to the conclusion that the early increase in the synthesis of ribosomal RNA is a non-specific cellular response to different injuring effects. In three-hr and 12-hr regenerating livers, however, irradiation led to a suppression of the synthesis of pre-messenger RNA independent of the time of irradiation. It is suggested that in normal liver, only those types of messenger RNA are synthesized which are necessary for production of proteins connected with the specialized functions of the hepatocytes. The stimulation of the synthesis of these messenger RNA species implies that irradiation stimulates the transcription of cistrons already operating at the time of irradiation. In regenerating liver, irradiation suppresses the production of only those types of messenger RNA whose synthesis begins as a result of reprogramming. This leads to the conclusion that the radiosensitive phase of messenger RNA biosynthesis is not the process of transcription *per se* but the switching on of new genes transcribing new types of messenger RNA.

0789 THE EFFECT OF NEPHRECTOMY ON THE INCIDENCE OF BREAST CARCINOMA IN IRRADIATED PARABIOSED RATS. (Eng.) Brown, C. E. (N. Engl. Deaconess Hosp., Boston, Mass.); Chute, R. N.; Porter, M. W.; Warren, S. *Cancer Res.* 35(1):37-44; 1975.

The effect of nephrectomy on the incidence of breast carcinoma in irradiated parabiosed rats was investigated in a study of the interplay of endogenous hormones. Forty-six pairs of inbred virgin female NEDH rats were joined in parabiosis when about 40 days old. These were divided into three groups: (1) the right partner of 32 pairs received 1000 rads total-body irradiation after which a kidney was removed from each partner; (2) a kidney was removed from each partner of ten pairs after which the right partner received 1000 rads total-body irradiation; and (3) a kidney was removed from each partner of four pairs, and no irradiation was given (control group). Irradiation and nephrectomies were performed within 13 weeks of parabiosis. Of the 42 experimental pairs of parabiosed rats, adenocarcinomas occurred in the breasts of 21 of the nonirradiated partners. One pair had an adenocarcinoma in both partners, and in three other pairs, the irradiated partners had breast carcinoma. Nodular breast tumors appeared 5-14 months postnephrectomy, many of which became hemorrhagic, and some of which ulcerated. The four breast carcinomas were well-differentiated. The ovaries of the nonirradiated partners with breast carcinoma were consistently enlarged, riddled with cysts lined with either granulosa cells, luteinized cells, or mixtures of both in single or multiple layers. Failure of resolution of the lutein cysts suggested that progesterone secretion was continuing during the early stages of breast carcinoma development. In hormonal analyses, estradiol and progesterone were higher in the blood of the nonirradiated partners than in the irradiated partners, while follicle-stimulating hormone was higher in the irradiated partner. A nearly 4-fold increase in the average progesterone concentration occurred in the nonirradiated partners with breast carcinoma compared to their partner's average. Average prolactin levels in the nonirradiated partners with breast carcinoma exceeded those of their irradiated partners. It is suggested that interference with excretion of endogenous gonadotropins by the kidney or stress-induced suppression of prolactin inhibition may have helped sustain a prolactin-progesterone increase with a subsequent increase in breast carcinoma.

0790 DNA REPAIR IN TUMOR CELLS FROM THE VARIANT FORM OF XERODERMA PIGMENTOSUM. (Eng.) Robbins, J. H. (Natl. Cancer Inst., Bethesda, Md.); Kraemer, K. H.; Flaxman, B. A. *J. Invest. Dermatol.* 64(3):150-155; 1975.

UV-induced ^{3}H-thymidine incorporation in cultures of basal cell carcinoma cells from a patient with variant xeroderma pigmentosum (XP) and from a patient with XP was studied. The latter patient had classical XP with decreased UV-induced ^{3}H-thymidine incorporation. The patient with variant XP had clinically classical disease, but did not show any defect in the rate of UV-induced ^{3}H-thymidine incorporation into his lymphocytes. Tumor cells and fibroblasts from both patients were irradiated in phosphate-buffered saline for 150 sec with a 2537 Å germicidal lamp at an incident flux of approximately 1-2 erg/mm^2/sec. The cells were incubated for three hr with ^{3}H-thymidine. The irradiated fibroblasts and tumor cells from the patient with variant XP had as much UV-induced ^{3}H-thymidine uptake as did normal fibroblasts. In contrast, a very

small amount of ^{3}H-thymidine was incorporated into basal cell carcinoma cells from the patient with XP whose lymphocytes and fibroblasts had been shown previously to have from 15-25% of the normal rate of UV-induced ^{3}H-thymidine incorporation. It is concluded that there is no evidence that the tumor cells of the patient with variant XP arose from a cell or cells which had markedly reduced UV-induced ^{3}H-thymidine incorporation. According to previously published reports, XP variant cells, including cells from the patient investigated in this experiment, have a defect in the postreplication repair process. It is suggested that cells from the patient with variant XP probably have a DNA repair defect, and that this defect may well be the cause of the clinical manifestations of XP.

0791 XERODERMA PIGMENTOSUM VARIANTS HAVE DECREASED REPAIR OF ULTRAVIOLET-DAMAGED DNA. (Eng.) Day, R. S. (Natl. Cancer Inst., Bethesda, Md.). *Nature* 253(5494):748-749; 1975.

A sensitive host-cell reactivation technique was used to estimate DNA repair in fibroblasts from patients belonging to all five known variant kindreds of xeroderma pigmentosum. In this study all five variant strains manifested similar degrees of decreased host-cell reactivation of UV-irradiated adenovirus 2, ranging from 57 to 64% of normal. While all variants are unable to repair UV irradiation-damaged DNA as well as normal cells, it is not yet known which repair process is defective.

0792 POSSIBLE DEVELOPMENT OF SKIN BASALIOMA AS A RESULT OF EXTERNAL α RADIATION. (Cze.) Sevcova, M. (Inst. Hyg. Epidemiol., Prague, Czechoslovakia); Dandy, J. *Cesk. Dermatol.* 50(2): 129-134; 1975.

0793 THYMOCYTE ELECTROPHORETIC MOBILITY DURING IRRADIATION-INDUCED THYMIC LEUKEMOGENESIS [abstract]. (Eng.) Brown, R. C. (Univ. North Carolina, Chapel Hill); Kostyu, J. A.; Kilgore, A. *Fed. Proc.* 34(3):833; 1975.

0794 PAROTID TUMOR AND THYROID CANCER: SIMULTANEOUS OCCURRENCE AFTER IRRADIATION OF THE NECK IN CHILDHOOD. (Eng.) Becker, F. O. (Presbyterian-St. Luke's Hosp., 1753 Congress Parkway, Chicago, Ill. 60612); Economou, S. G. *J.A.M.A.* 232(5):512-514; 1975.

0795 ACTIVITY ON γ-IRRADIATED AND 7-BROMOMETHYL-12-METHYLBENZ[A]-ANTHRACENE TREATED DNA OF ENDONUCLEASE II OF *E. COLI* [abstract]. (Eng.) Kirtikar, D. (Case West. Reserve Univ., Cleveland, Ohio); Goldthwait, D. A. *Fed. Proc.* 34(3):515; 1975.

0796 MUTAGENIC EVALUATION OF AN ALCOHOLIC EXTRACT FROM γ-IRRADIATED POTATOES. (Eng.) Levinsky, H. V. (Bio-Res. Lab. Ltd., Pointe Claire, Quebec, Canada); Wilson, M. A. *Food Cosmet. Toxicol* 13(2):243-246; 1975.

0797 LOCALISATION OF PLUTONIUM IN MOUSE TESTES. (Eng.) Green, D. (Med. Res. Counc. Radiobiol. Unit., Harwell, Didcot, Oxfordshire OX11 ORD, UK); Howells, G. R.; Humphreys, E. R.; Vennart, J. *Nature* 255(5503):77; 1975.

0798 THE EFFECTS OF γ-RADIATION ON SOLUTIONS OF ACETYLCHOLINESTERASE [abstract]. (Eng. Nayar, G. N. A. (Inst. Med. Sci., Banaras Hindu Univ., Varanasi, India); Srinivasan, S. *Biophys. J.* 15(2):134a, 1975.

0799 ULTRASTRUCTURE OF NUCLEI IN IRRADIATED TUMOR CELLS [abstract]. (Eng.) Overgaard, J. (Inst. Anat., Univ. Aarhus, Denmark). *J. Ultrastruct. Res.* 50(3):389-390; 1975.

0800 HEPATOMA AFTER THOROTRAST [abstract]. (Eng.) Mann, N. S. (Univ. of Louisville Sch. of Medicine, Louisville, Ky.); Chaudhry, A.; Thaller, S.; Sachdev, A. *Gastroenterology* 68: (4/Part 2):945; 1975.

See also:

* (Rev): 605, 621, 631, 632, 633, 634
* (Chem): 665, 710, 782
* (Immun): 903, 949

0801 PRODUCTION OF MACROPHAGE MIGRATION INHIBITORY FACTOR(S) (MIF) BY VIRUS-TRANSFORMED CELLS. (Eng.) Poste, G. (Dept. Experimental Pathology, Roswell Park Memorial Inst., Buffalo, N.Y. 14203). *Exp. Cell Res.* 92(2):283-290; 1975.

The production of migration inhibitory factor-like components by non-lymphoid rodent cells transformed by simian virus 40 (SV40), polyoma virus (PY), and Rous sarcoma virus (RSV) was examined. Transformed cell lines used were SV3T3, PY-BHK, RSV-BHK, PY-NIL, RSV-NIL, and rat hepatoma RLT. Supernatants were harvested from cell cultures in which the growth medium had been changed 24 hr before harvesting and replaced either with serum-free medium or medium containing guinea pig or fetal calf serum. Supernatants were centrifuged at 1,000 x *g* for 20 min to remove suspended cells, passed through a Millipore filter, and then tested for migration inhibitory factor. Cell-free supernatants harvested from the transformed cell lines caused significant inhibition of the migration of guinea pig macrophages from capillary tubes. Similar cell-free supernatants harvested from the untransformed and transformed cell populations at different stages of growth failed to reveal any relationship between migratory inhibitory factor and the rate of cell division. Comparison of the migration inhibitory factor produced by virus-transformed cells with the migration inhibitory factor derived from peripheral lymphocytes, stimulated *in vitro* by phytohemagglutinin, revealed that they had similar molecular weight (25,000) and heat stability, and were both inhibited by α-fucose and lotus agglutinin. Incubation of migration inhibitory factor-containing cell-free supernatants from transformed cells with the protease inhibitors, pancreatic trypsin inhibitor, soybean trypsin inhibitor and diisopropylfluorophosphate, eliminated the migration inhibitory factor activity, suggesting that this factor may be a trypsin-like protease. However, the lack of effect of other trysin inhibitors, such as ovomucoid and *N*-α-tosyllysine chloromethyl ketone, indicates that it differs from trypsin in at least some properties. It is suggested that the migration inhibitory factor may represent a potentially important mechanism in modifying the cell-mediated immune response of the host in the vicinity of tumors.

0802 INCREASED OUABAIN-SENSITIVE $^{86}Rb^+$ UPTAKE AND SODIUM AND POTASSIUM ION-ACTIVATED ADENOSINE TRIPHOSPHATE ACTIVITY IN TRANSFORMED CELL LINES. (Eng.) Kimelberg, H. K. (Albany Medical Coll., Albany, N. Y. 12208); Mayhew, E. *J. Biol. Chem.* 250(1):100-104; 1975.

The effects of viral transformation on K^+ uptake and on sodium and potassium ion-activated ATPase [(Na^+ + K^+)-ATPase] activity in Balb/c 3T3 and baby hamster kidney (BHK) cells were studied. Cells were prepared for uptake studies by adding 0.2 ml of ouabain at a concentration of 1 or 5 mM. After a 15 min incubation, approximately 5 μCi of $^{86}Rb^+$ as RbCl was added giving 2-3 x 10^5 cpm. ATPase determinations were made on cells collected by trypsinization or directly in culture tubes. During the log phase of growth both the active, ouabain-sensitive K^+ uptake and the (Na^+ + K^+)-ATPase activity of simian virus (SV) 40-transformed 3T3 cells were 2.5- and 5.5-fold higher, resp., than in untransformed 3T3 cells. A similar higher active K^+ uptake was found for Rous sarcoma virus and SV40-transformed BHK cells compared with untransformed BHK cells. The active K^+ uptake in SV40-transformed 3T3 and normal 3T3 cells decreased when the growth rate of both cell types diminished. Reduction in ouabain-sensitive ATP hydrolysis occurred later, when decreases in cell viability were seen. Arrhenius plots of the (Na^+ + K^+)-ATPase activity of SV40-3T3 cells indicated a discontinuity at 24 C. No similar discontinuity was indicated for 3T3 cells. It is suggested that the increased activity in transformed cells may be related to increased phospholipid fatty acyl chain fluidity.

0803 A HISTOCHEMICAL STUDY OF ACID PHOSPHATASE IN NORMAL AND VIRUS-TRANSFORMED CULTURED FIBROBLASTS. (Eng.) Dawson, A. L. (Thames Polytechnic, Woolwich, England); Beadle, D. J.; Livingston, D. C.; Fisher, S. W. *Histochem. J.* 7(1):77-84; 1975.

A histochemical technique was used to study the distribution of acid phosphatase in normal BHK21C13 and polyoma virus-transformed BHK21JI hamster cells and normal 3T3 and Simian virus-transformed SV403T3 mouse cells. The main site of acid phosphatase activity in normal and transformed hamster cells was in small, membrane-bound lysosomes. Autophagic vacuoles were more numerous in normal than in transformed hamster cells, and many of the Golgi bodies of both cell types showed activity. The normal 3T3 cells had many more lysosomes and autophagic vacuoles than the SV403T3-transformed cells. The Golgi apparatus in the normal cells was also more developed than in the transformed cells. The SV403T3 cells often contained extremely large autophagic vacuoles, and a few cells had a distinct surface coat. It is concluded that viral transformation has little or no effect on the distribution of acid phosphatase in the cell lines studied.

0804 STUDIES ON REVERSE TRANSCRIPTASE OF RNA TUMOR VIRUSES. I. LOCALIZATION OF THERMOLABILE DNA POLYMERASE AND RNase H ACTIVITES ON ONE POLYPEPTIDE. (Eng.) Verma, I. M. (Tumor Virology Lab., Salk Inst., San Diego, Calif. 92112). *J. Virol.* 15(1):121-126; 1975.

Purified reverse transcriptase from avian myeloblastosis virus or Rous sarcoma virus consists of two subunits of average molecular wt of 100,000 and 60,000. The lower-molecular-wt subunit, α, has been isolated from avian myeloblastosis virus, Rous sarcoma virus, and a temperature-sensitive mutant of Rous sarcoma virus, LA337. Subunit α manifests both the DNA polymerase and RNase H activities associated with purified reverse transcriptase of avian RNA tumor viruses. The thermal inactivation of these enzymatic activities of α subunit from LA337 was compared with the isolated α subunit from the wild-type virus. Approximately 4.5 mg of purified

virions from wild-type Rous sarcoma virus were lysed with Nonidet P-40, and DNA polymerase was purified by column chromatography on DEAE-Sephadex A-25. Enzymatic assays were performed and the peak fractions with enzymatic activities were dialyzed overnight against a buffer containing 50 mM Tris-hydrochloride, 0.1 mM EDTA, 0.1 M β-mercaptoethanol, 20% glycerol, and 0.15% Nonidet P-40. The dialyzed enzyme was further purified on a phosphocellulose column. RNA-directed DNA synthesis was assayed using poly(C)-oligo(dG) as template primer; DNA-directed DNA synthesis was assayed using poly(dC)-oligo(dG) as template primer. The results show that both DNA polymerase and RNase H activities associated with the α subunit of LA337 are 5-7 times more thermolabile than the corresponding α subunit from the wild-type virus. It is concluded that both the polymerase and nuclease activities reside on the same polypeptide chain, and that at least the lower-molecular-wt subunit α is coded by the viral RNA.

0805 POST-TRANSCRIPTIONAL RESTRICTION OF HUMAN ADENOVIRUS EXPRESSION IN MONKEY CELLS. (Eng.) Eron, L. (Natl. Inst. Child Health Hum. Dev., Bethesda, Md.); Westphal, H.; Khoury, G. *J. Virol.* 15(5):1256-1261; 1975.

The biological activity of adenovirus type 2 (Ad2) mRNA in cell-free protein-synthesizing systems was investigated. Two cell lines of African green monkey kidney cells (CV-1 and BSC-1) and Vero (a Simian cell line) were used. When infected simultaneously with Ad2 and helper simian virus 40 (SV40) all three cell lines produced structural Ad2 proteins. However, Vero cells appeared independent of the SV40 helper function in their ability to produce Ad2 proteins. Analysis for endogenous SV40 DNA was negative. When input multiplicity of Ad2 was increased from 5 to 50, the BSC-1 line contained comparable amounts of Ad2 structural components regardless of coinfection with SV40. At low multiplicity of infection, hexon synthesis in CV-1 and BSC-1 line was strictly dependent on SV40 helper; this was not the case for Vero cells. Polysomal pellets from enhanced and unenhanced cells were found to contain near equal amounts of pulse-labeled poly(A)-Ad2 RNA and, in both cases, cosedimented with 80S polysomal structures. Thus, the synthesis and attachment of Ad2 RNA to ribosomes is restricted in unenhanced cells. The polypeptide comigrated with all major adenovirion polypeptides in sodium dodecyl sulfate polyacrylamide gel and no difference was evident in relative quantities which were directly related to RNA input. A positive host cell factor may regulate the expression of Ad2 after the association of mRNA with microsomes, but prior to the initiation of protein synthesis.

0806 STRUCTURE OF THE DNA OF THE ADENOVIRUS 7-SIMIAN VIRUS 40 HYBRID, E46$^+$, BY ELECTRON MICROSCOPY. (Eng.) Kelly, T. J., Jr. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.). *J. Virol.* 15(5):1267-1272; 1975.

The structure of the DNA of adenovirus 7-simian virus 40 hybrid (E46$^+$) was studied using heteroduplex methods and electron microscopy. To determine the sequences in the E46$^+$ hybrid, linear simian virus 40 (SV40) DNA strands were prepared and hybridized to the adenovirus 7 (Ad7)/(E46$^+$) heteroduplexes, giving rise to a duplex circle with two single-stranded tails. The tails measured 0.11 and 0.29 fractional SV40 lengths. This indicated that the circles in the duplexes contained the segment SV40 genome extending from SV40 map position about 0.11-0.71 (clockwise). The distribution of crude protein was random, indicating tandem organization of the type 123412. Using an Ad7/E46$^+$/*Hin*III-C heteroduplex, the *Hin*III-C fragment represented 20.5% of the SV40 genome and lay in positions 0.655 and 0.860. A part of the fragment was contained within the SV40 DNA segment of E46$^+$: the remainder was deleted. Hybridization of *Hin*III-C fragment to SV40 DNA produced a short duplex segment with a single-stranded tail emerging at the SV40 deletion point (0.22 SV40 length from left end of SV40 DNA). Examining the Ad7/E46$^+$/*Hin*III-C heteroduplexes gave the orientation of the SV40 DNA sequences in E46$^+$ from SV40 map position 0.49 to SV40 map position 0.65 and repeated in tandem. This heteroduplex was also examined electron microscopically, which confirmed the SV40 sequences of E46$^+$. Elucidating the mechanism of differential translation of SV40 DNA may aid the understanding of the control of SV40 gene expression in transformed cell

0807 TEMPERATURE-SENSITIVE CELL MUTATIONS THAT INHIBIT ADENOVIRUS 2 REPLICATION. (Eng.) Nishimoto, T. (Washington Univ. Sch. Med., St. Loui Mo. 63110); Raskas, H. J.; Basilico, C. *Proc. Natl Acad. Sci. USA* 72(1):328-332; 1975.

The role of the host cell in regulating the multipl cation of an oncogenic DNA virus was studied. Five temperature-sensitive (ts) growth mutants of the hamster kidney cell line BHK-21 were tested for the ability to support human adenovirus 2 multiplication at 39 C and 33 C. Virus was purified by CsCl density gradient centrifugation. Infectious virus was determined by plaque assay on monolayers of hum KB cells. DNA synthesized by infected and mock-inf ted cultures was labeled by incubating cultures wit (^{3}H)thymidine (20 μCi/ml, 6.7 Ci/mM) for 3 hr intervals. Wild-type BHK-21 and mutants ts 422E and ts BCH yielded comparable amounts of virus at 33 C and 39 C. In three other mutants, ts T22, ts T23, and ts AF8, virus production at 39 C was reduced to about 1% of that at 33 C. Virus yield in the three mutants was not reduced because of a delay in virus production; for all cells tested maximal virus yiel at 39 C was obtained by 40-50 hr after infection. Normal yields of infectious virus were not obtained from ts AF8 even with a very high multiplicity of infection. The virus yield from ts T22 and ts T23 was multiplicity-dependent. Shift-up experiments showed that in ts AF8, a cell cycle mutant which at 39 C becomes arrested in G1, virus multiplication was thermosensitive for the first 40 hr of infectio In ts T22 and ts T23, the thermosensitivity was onl for the first 3-4 hr of the infection. In all thre mutants, viral DNA synthesis was reduced by at leas 95% at the higher temperature. It is suggested tha

the cell function specified by the ts AF8 is required for the early period of adenovirus 2 replication, after virus entry into the cell but before the onset of viral DNA replication.

0808 SEQUENCE RELATIONSHIPS BETWEEN ADENOVIRUS 2 EARLY RNA AND VIRAL RNA SIZE CLASSES SYNTHESIZED AT 18 HOURS AFTER INFECTION. (Eng.) Tal, J. (Washington Univ. Sch. Med., St. Louis, Mo.); Craig, E. A.; Raskas, H. J. *J. Virol.* 15(1): 137-144; 1975.

Synthesis of cytoplasmic viral RNA was studied during infection of cultured human (KB) cells with adenovirus 2 to determine the sequence relationship between the major cytoplasmic size classes synthesized late in infection and those viral RNAs present at early times. Exponentially growing KB suspension cultures were concentrated to 1.2 x 10^7 cells/ml and infected with adenovirus 2 (40 plaque forming U/cell). After a one-hr adsorption period, the culture was diluted to 3 x 10^5 cells/ml. Portions of KB cells infected with adenovirus 2 were concentrated 4-fold and labeled with [^{3}H]uridine for two hr. Cytoplasmic RNA was purified from the labeled culture samples and a portion was used to prepare poly(A)-containing molecules. Hybridization to viral DNA increased as the infection progressed. At six hr, before viral DNA synthesis began, 5% of the poly(A)-containing RNA hybridized to viral DNA; by 12 hr and at later times, more than 80% was virus-specified. At 18 hr after infection, four major size classes of cytoplasmic viral RNA were identified among the poly(A)-containing molecules. These sizes migrated as 27 S, 24 S, 19 S and 12 to 15 S in polyacrylamide gels. The three larger size classes could also be identified in denaturing formamide gels. Hybridization of the 27 S, 24 S and 19 S viral RNAs was not inhibited by RNA harvested from cells at early times in infection, indicating that these three major RNAs must code for late viral proteins such as those required for virion assembly. Hybridization of the 12 to 15 S RNA was partially inhibited by RNA from cultures harvested at early times, suggesting that, in this size class, some of the RNA labeled at 18 hr codes for early viral proteins.

0809 DNA BINDING PROTEIN IN THE CYTOPLASM AND IN A NUCLEAR MEMBRANE COMPLEX ISOLATED FROM UNINFECTED AND ADENOVIRUS 2 INFECTED CELLS. (Eng.) Shanmugam, G. (St. Louis Univ. Sch. Medicine, Mo. 63110); Bhaduri, S.; Arens, M.; Green, M. *Biochemistry* 14(2):332-337; 1975.

The DNA-binding proteins in a nuclear membrane fraction that can synthesize DNA *in vitro* and in the cytoplasm of adenovirus infected and uninfected cells were isolated and characterized. Suspension cultures of human KB cells infected with human adenovirus 2 (100 plaque-forming U/cell) were treated with 25 μg/ml of arabinosylcytosine and then labeled with (^{3}H)leucine from 6-24 hr postinfection. Uninfected cells were treated similarly and labeled with (^{14}C)-leucine. The ^{3}H-labeled and ^{14}C-labeled proteins isolated from the cytoplasm were mixed, as were corresponding proteins isolated from the membrane complex. DNA-binding proteins were selected by adsorption in 0.05 M NaCl to single-stranded DNA-cellulose columns and fractionated by elution with 0.15, 0.4, 0.6, and 2 M NaCl. A major fraction of the labeled proteins from the nuclear membrane complex (50-60%) and a small amount from the cytoplasm (10-20%) bound to DNA cellulose in 0.05 M NaCl. Larger quantities of DNA binding proteins were present on the nuclear membrane complex and in the cytoplasm of infected cells than in the corresponding fractions from uninfected cells. The electrophoretic profile of the 0.15 and 0.4 M NaCl eluates from the nuclear membrane complex revealed a heterogeneous mixture of polypeptides in infected and uninfected cells. The 0.6 M eluate contained two major components of molecular wt 75,000 and 45,000 that were present only in infected cell preparations. These two proteins were also present in the same eluate from the cytoplasm of infected cells. Two major cell specific components of molecular wt 40,000 and 15,000-17,000 were present in the 2 M eluate of the membrane complex. One major cell-specific protein of molecular wt 33,000 was present in the 0.15 and 0.4 M eluate of the cytoplasm of infected and uninfected cells. Analysis of cells labeled in the absence of arabinosylcytosine indicated that synthesis of the DNA binding proteins of molecular wt 75,000 and 40,000 begins early after infection prior to the onset of viral DNA replication. It is suggested that the two DNA binding proteins in adenovirus 2-infected cells reflect the expression of the parental viral genome.

0810 SPECIFIC CLEAVAGE OF ADENOVIRUS-ASSOCIATED VIRUS DNA BY RESTRICTION ENDONUCLEASE R·*Eco*RI--CHARACTERIZATION OF CLEAVAGE PRODUCTS. (Eng.) Carter, B. J. (Natl. Inst. Arthritis Metab. Dig. Dis., Bethesda, Md.); Khoury, G. *Virology* 63(2):523-538; 1975.

The cleavage of adenovirus-associated virus type 2 (AAV2) DNA by the restriction endonuclease R x *Eco*RI was investigated using neutral and alkaline sucrose sedimentation, electrophoresis in composite agarose-acrylamide gels, and reassociation kinetics. AAV2 and adenovirus type 2 were propagated by coinfection of KB-3 cells. Cells were harvested at 40-48 hr post-infection, and the virions were purified by sonication and digestion with trypsin and sodium deoxycholate, followed by three cycles of preparative centrifugation. Viral DNA was extracted from purified virions using an enzymatic digestion procedure. Bromodeoxyuridine-substituted DNA was used to obtain specific duplex fragments of AAV DNA that could then be separated into complementary strands. Preparations of bromodeoxyuridine-substituted or non-substituted DNA, labeled with either ^{3}H or ^{32}P, were used interchangeably and gave similar results. For cleavage of DNA, reaction mixtures contained 0.1 M Tris-HCl, 5 mM MgCl, up to several μg of DNA, and R x *Eco*RI restriction enzyme (1 U/50 mg of DNA). The DNA duplexes were cleaved at two specific sites to give three fragments, A, B and C, equivalent in size to 57.2, 38.2 and 4.6%, resp., of the intact genome. The distribution of isotope in AAV DNA

randomly labeled with ^{32}P, together with molecular weight estimates, indicated that all three fragments were produced in equimolar proportions, and that each probably contained a unique region of the duplex genome. Because of some uncertainty in the molecular weight estimates of C, it is possible that this fragment might represent two fragments of 2.5% of the genome rather than one 5% fragment. Cleavage of AAV DNA yielded, in addition to fragments A, B, and C, two components AB and AB', both of which consisted of equimolar amounts of A and B strands present in structures which depended, at least in part, upon annealing of cohesive ends. The remaining portion of both AB and AB' may consist of branched molecules and single-stranded regions. These results support a previously published cleavage model which suggests the presence of cohesive ends in AAV duplex DNA, apparently resulting from a limited permutation such that the starting point of each strand may vary within a 6% region of the genome sequence.

0811 DIFFERENTIAL ACCUMULATION OF VIRUS-SPECIFIC RNA DURING THE CELL CYCLE OF ADENOVIRUS-TRANSFORMED RAT EMBRYO CELLS. (Eng.) Hoffmann, P. R. (Dep. Biol. Sci., Columbia Univ., New York, N.Y.); Darnell, J. E., Jr. *J. Virol.* 15(4):806-811; 1975.

The possibility of the occurrence of a 'built-in' regulation, related to the cell cycle, for virus-specific proteins or virus-specific RNA in transformed cells was investigated. Employing adenovirus type 2-transformed rat embryo cells (strain 8617), the relative amount of [^{3}H] uridine incorporation into virus-specific RNA in the nucleus and in the cytoplasm of the adenovirus-transformed cells was measured at various times in the cell cycle. Using a labeling time of 60 min, the total virus-specific radioactivity incorporation in a 1 hr period showed a pronounced fluctuation during the 12 hr cell cycle in synchronized adenovirus type 2-transformed rat cells. Cells in the first three labeling periods accumulated 3-4 times as much labeled virus-specific RNA as cells in the next two periods, followed by a resumption after completion of mitosis. The fluctuation occurred with both nuclear and cytoplasmic samples, with a slightly more marked drop before mitosis in the cytoplasmic RNA than in the nucleus. Essentially similar results were obtained in cells treated with 0.0225 μg/ml actinomycin D. The experiments established that proportionately more labeled Ad-2RNA accumulates both in the nucleus and in the cytoplasm during early and middle S phase than during G2 and late S phase. In addition, blocking the entrance of cells into S phase by excess thymidine prevented the previously observed increased accumulation of viral-specific RNA. Additional tests with hydroxyurea-treated cells revealed that not only the initiation of DNA synthesis, but its continuation appeared important for the increase in Ad-2 RNA labeling during the S phase. The results indicate that a specific type of RNA does not accumulate equally throughout the cell cycle, although fluctuation is at most 3-fold; also, that at all times the relative accumulation of nuclear and cytoplasmic virus-specific RNA is parallel.

0812 THE 3'-TERMINAL NUCLEOTIDE SEQUENCES OF ADENOVIRUS TYPES 2 AND 5 DNA. (Eng.) Steenbergh, P. H. (Lab. Physiol. Chem., State Univ. Utrecht, Netherlands); Sussenbach, J. S.; Roberts, R. J.; Jansz, H. S. *J. Virol.* 15(2):268-272; 1975.

The short nucleotide sequence at the 3'-termini of adenovirus types 2 and 5 DNA were determined using T4 DNA polymerase. Adenovirus 2 or 5 DNA was incubated in the presence of T4 DNA polymerase and a single α-[^{32}P]deoxyribonucleoside triphosphate (dNTP) with or without one of the other unlabeled dNTPs. For both types of DNA the incorporation of [^{32}P]deoxyguanosine monophosphate (dGMP) was not influenced by the presence of the other dNTPs, whereas incorporation of [^{32}P]deoxythymidine monophosphate (dTMP) was inhibited only by deoxyguanosine triphosphate (dGTP). The incorporation of [^{32}P]deoxyadenosine AMP was inhibited by deoxyguanosine triphosphate and deoxythymidine triphosphate, whereas the incorporation of [^{32}P]deoxycytidine monophosphate was affected by all three remaining dNTPs. The presence of identical relative positions at both 3'-termini was verified for adenovirus 5 DNA by analyzing the incorporation of radioactivity at each terminus separately. After incubation of adenovirus 2 or 5 DNA in the presence of T4 DNA polymerase and a single α-[^{32}P]dNTP, the DNA was isolated and the nearest neighbor of the incorporated [^{32}P]dNMP was determined. In all cases the transfer of ^{32}P to one of the four 3'-mononucleotides was 60-80%, whereas the remaining 20-40% of the radioactivity was divided among the other three nucleotides. This indicates that the same dinucleotide sequences are present at both molecular ends. Combination of these data with the relative positions ...C...A...t...G$^{3'}$ leads to the conclusion that the 3'-terminal sequences of adenovirus 2 and 5 DNA are ...pCpC...pGpApTpG$^{3'}$.

0813 TRANSCRIPTION OF THE GENOME OF ADENOVIRUS TYPE 12. I. VIRAL mRNA IN ABORTIVELY INFECTED AND TRANSFORMED CELLS. (Eng.) Ortin, J. (Inst. Genetics, Univ. Cologne, West Germany); Doerfler, W. *J. Virol.* 15(1):27-35; 1975.

The kinetics of appearance of the polysome-associated adenovirus type 12 messenger (m)RNA in baby hamster kidney (BHK) 21 cells is described. In cells abortively infected with adenovirus type 12, virus-specific RNA was detected 5-7 hr after infection. The amount of RNA reached a maximum between 10 and 12 hr after infection and continued to be synthesized at a reduced level until 48-50 hr postinfection. In BHK-21 cells transformed by adenovirus type 12, 0.26% of the polysome-associated mRNA was virus-specific. The size of the virus-specific mRNA isolated from polysomes of cells abortively infected with, or transformed by, adenovirus type 12 was determined by electrophoresis in polyacrylamide gels in 98% formamide. These conditions eliminated secondary structure or aggregation of RNA. In abortively infected BHK-21 cells, viral mRNA size classes of molecular wt 0.9 x 10^6 and 0.65 x 10^6 to 0.67 x 10^6 predominated. A minor fraction of 1.5 x 10^6 daltons was consistently found and increased with time after infection. Twen-

ty-four to 26 hr postinfection, viral mRNA of 1.9 x 10^6 daltons was also observed. The size distribution of adenovirus type 12-specific mRNA from transformed cells was similar to that in abortively infected cells, except that the relative amount of the viral mRNA fraction of 1.5 x 10^6 daltons was much higher. It could not be determined whether the transcripts in abortively infected and transformed BHK-21 cells are derived from integrated viral genomes or from free viral DNA.

0814 PURIFICATION AND CHEMICAL CHARACTERIZATION OF THE MAJOR GLYCOPROTEIN OF AVIAN MYELOBLASTOSIS VIRUS. (Eng.) Porter, W. H. (Univ. Tennessees Memorial Res. Center, Knoxville, Tenn. 37920); Winzler, R. J. *Arch. Biochem. Biophys.* 166(1):152-163; 1975.

The purification, and the carbohydrate and amino acid composition of the major avian myeloblastosis virus (AMV) glycoprotein are described. The glycoprotein was purified by gel filtration on a Sepharose 4B column in the presence of 6 M guanidine hydrochloride followed by dialysis against distilled water and extraction with chloroform-methanol. The AMV glycoprotein remains soluble in the aqueous phase while contaminating proteins precipitate upon dialysis with distilled water or after treatment with chloroform-methanol. Carbohydrate, represented by glucosamine, mannose, galactose, fucose, and sialic acid, constitutes 40% of the wt of AMV glycoprotein. Glucosamine is the major carbohydrate component whereas fucose and sialic acid are present in relatively low amounts. Amino acid analysis indicates a relatively high content of aspartic and glutamic acid, serine, threonine, and glycine. Based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a molecular wt value of 77,500 ± 500 was determined for AMV glycoprotein.

0815 MALIGNANT TRANSFORMATION OF HAMSTER KIDNEY CELLS BY BK VIRUS. (Eng.) Portolani, M. (Inst. Microbiol., Univ. Bologna, Italy); Barbanti-Brodano, G.; La Placa, M. *J. Virol.* 15(2):420-422; 1975.

The neoplastic transformation of hamster kidney cells by BK virus, a new human papovavirus, is reported. BK virus was grown in Vero cells and titrated in human embryonic fibroblasts by the fluorescent antibody focus assay and by hemagglutination of human type O erythrocytes. Primary hamster kidney cells obtained by trypsinization of kidneys from 72-hr-old baby hamsters were infected with two fluorescent antibody focus-forming U/cell. Transformed HKBK cells produced BK virus T antigen and induced tumors in hamsters that developed antibodies to BK virus T antigen. BK virus was rescued from HKBK cells by Sendai virus-assisted fusion with permissive cells. One out of six cell lines derived from HKBK cell-induced tumors showed the same characteristics as HKBK cells. BK virus could not be detected by hemagglutination in cell homogenates from fused cultures unless the cultures were subcultured (six to ten times) and the cell homogenate treated with receptor-destroying enzyme. Virus activity was completely inhibited by a 1:100 dilution of specific guinea pig serum to BK virus. The low frequency of rescue, in spite of the very good efficiency of fusion, was probably due to the high number of defective particles present in the virus inoculum. It is likely that very few cells were infected and transformed by virions containing a fully infectious genome; most of the cells were probably infected by defective virus particles. This may explain why essentially all BK virus-transformed cells produced T antigen, whereas only a few of them were inducible when fused with permissive cells. Alternatively, a large number of transformed cells may contain a complete viral genome if the efficiency of the induction is low. This possibility is supported by the observation that the activation of the viral genome in SV40-transformed cells fused with permissive cells is limited by the inefficient excision of viral DNA from cellular DNA.

0816 ISOLATION OF A B-TROPIC TYPE-C VIRUS FROM RETICULUM CELL NEOPLASMS INDUCED IN BALB/C MICE BY SJL/J TYPE-C VIRUS. (Eng.) Chang, K. S. S. (Natl. Cancer Inst., Bethesda, Md.); Aoki, T.; Law, L. W. *J. Natl. Cancer Inst.* 54(1):83-87; 1975.

Newborn thymectomized BALB/c mice were inoculated with Dl-murine leukemia virus (MuLV), isolated from a reticulum cell neoplasm (RCN-B) and propagated in mouse embryo cell cultures, to determine the pathogenic potential of Dl-MuLV. Thirty percent of the mice developed RCN-B within five months. High titers of B-tropic type-C viruses (El-MuLV) were isolated from the spleen and lymph nodes of all eight of the tumor-bearing animals tested. Low titers of N- or B-tropic viruses were found in the mice that did not develop RCN-B and only N-tropic viruses were found in uninoculated controls. Nonthymectomized untreated controls showed no virus at all. Antisera were used to type the viral envelope antigen (VEA) of the Dl-MuLV. The VEA of Dl-MuLV was distinct from those of El-MuLV. The El-MuLV VEA was also distinct from xVEA and Gross-VEA, but showed some cross reaction to AKR-MuLV-VEA. It is suggested that Dl-MuLV is related to RCN-B oncogenesis in BALB/c mice and that the El-MuLV is selectively activated in RCN-B cells.

0817 ISOLATION OF INFECTIOUS XENOTROPIC MOUSE TYPE C VIRUS BY TRANSFECTION OF A HETEROLOGOUS CELL WITH DNA FROM A TRANSFORMED MOUSE CELL. (Eng.) Scolnick, E. M. (Natl. Cancer Inst., Bethesda, Md.); Bumgarner, S. J. *J. Virol.* 15(5):1293-1296; 1975.

An endogenous xenotropic virus was isolated from Kirsten sarcoma virus-transformed BALB/c (Ki-BALB) mouse cells. The DNA from this virus was extracted and used to transfect the mink fibroblast cell line (CCL64) using the calcium phosphate method. As early as 13 days after transfection, a low level of viral polymerase was detected in two cultures and thereafter increased 50-fold in the supernatant of mink fibroblast cells. Transformed foci could

only be detected after one month from transfection. Treatment of the transfection solution with 5 μg DNase I/ml abolished the infectivity of DNA, indicating that murine type C virus can be obtained by transfection of mink cells with Ki-BALB DNA in the absence of transfection by Kirsten sarcoma viral genome. The host range of this virus was narrow, growing only in rabbit cell line (SIRC), in mink cell, rat fibroblast (NRK) and in duck cells. These data indicate that an endogenous xenotropic type C virus can be isolated from Ki-BALB cells by isolating the DNA from that cell and transfecting a permissive cell with the DNA preparation.

0818 MULTIPLE SPECIES-SPECIFIC AND INTERSPECIFIC ANTIGENIC DETERMINANTS OF A MAMMALIAN TYPE C RNA VIRUS INTERNAL PROTEIN. (Eng.) Davis, J. (Flow Lab., Inc., Rockville, Md.); Gilden, R. V.; Oroszlan, S. *Immunochemistry* 12(1):67-72; 1975.

P30 fragments generated by limited tryptic hydrolysis and p30 molecules modified reversibly by citraconylation were subjected to gel diffusion analyses to investigate the presence of multiple species-specific and interspecific antigenic determinants on this major internal group-specific protein of mammalian Type C viruses. Antisera specific for rat (RaLV), endogenous cat (RD-114), and mouse (MuLV) p30s were tested against untreated, citraconylated, and citraconylated-reversed p30s. In each case, citraconylated p30s were antigenically deficient; complete serological reactivity was recovered after reversal of the citraconylation reaction by incubation at pH 5.6 to 5.8 for 18 hr. The MuLV antiserum was separated into two distinct antibody populations by immunoabsorption. Two antisera which detect interspecies determinants as well as species-specific determinants were tested with heterologous p30s and citraconylated p30s. Both sera (anti-MuLV and anti-FeLV) showed the characteristic reaction of partial identity between homologous and heterologous p30s placed in adjacent wells. The heterologous citronylated p30s were still detected by these antisera, but were antigenically deficient with regard to intact heterologous p30s. At least six antigenic reactivities associated with MuLV p30 were visualized in the gel diffusion studies. Three of these determinants are species-specific and three interspecific. Citraconylated p30 were deficient in two of three species-specific reactivities and at least one of three interspecies reactivities. A tryptic fragment from mouse p30 contained one species-specific determinant not shared with citronylated p30 and lacked one interspecies determinant contained in citronylated p30. Citronylated p30 also contained a species-specific determinant not shared with the tryptic fragment. It is suggested that these determinants may be regions of defined sequence or may result from conformational requirements. Thus, the loss of both species-specific and interspecies reactivities in citronylated p30 molecules might be expected from conformational changes, while those determinants surviving this procedure and limited trypsinization might be definable in terms of primary structure.

0819 ISOLATION OF TYPE C VIRIONS FROM A NORMAL HUMAN FIBROBLAST STRAIN. (Eng.) Panem, S (Dept. of Pathology and Pediatrics, Univ. of Chicago Chicago, Ill. 60637); Prochownik, E. V.; Reale, F. R.; Kirsten, W. H. *Science* 189(4199):297-299; 1975.

HEL-12, type C virions, which are released spontaneously from a human fibroblast cell strain, were characterized morphologically and immunologically. HEL-12 cells were derived from the lungs of a spontaneously aborted 8-wk-old embryo. Type C virions were not detected after the cells were subcultured for a few generations, but were released after HEL-12 cell were serially cultured for six months. Electron microscopy revealed typical type C virions, both free in the extracellular space and budding from plasma membranes. The HEL-12 virions were purified by equilibrium centrifugation in sucrose gradients and examined for relatedness to Rickard feline leukemia viru (R-FeLV), simian sarcoma virus (SSV), and Rauscher mouse leukemia virus (R-MuLV) with the use of monospecific antisera to purified R-FeLV p27, SSV p30, and R-MuLV p30. A component of HEL-12 virions was precipitated by an antiserum to the interspecies-specific antigen (p30) of SSV. This suggests that HEL-12 virions are antigenically related to SSV. Antiserum to reverse transcriptase of gibbon ape leukemia virus inhibited the reverse transcriptase of HEL-12 virions and that of simian sarcoma virus, but had no effect on the corresponding enzymes of avian or murine RNA tumor viruses. It is concluded that this is the first documented isolation of type C virions from a diploid human cell strain.

0820 SEARCH FOR C-TYPE PARTICLES IN HUMAN NEOPLASIA. (Eng.) Szakacs, J. E. (St. Joseph's Hosp., Tampa, Fla. 33607); Szakacs, M. R. *Ann. Clin. Lab. Sci.* 5(1):14-22; 1975.

The salient biologic and morphologic characteristics of oncornaviruses are reviewed. The ultrastructure of replicating oncornaviruses is illustrated in detail. C-type particles, widespread in at least three orders of animals, were sighted in human sarcomas and leukemias. One case of infantile fibrosarcoma, which was surveyed for the presence of C-type particles, is presented. Tissue cultures deri from this tumor contained viral particles and had an elevated reverse transcriptase activity associated with the presence of 70S RNA. The particles were larger (125-150 nm) than those of the murine or avia type C particles. It is emphasized that electron microscopy is an important technique in virus identification since viruses are morphologic as well as biochemical entities.

0821 INFLUENCE OF INTERFERON ON THE SYNTHESIS O VIRUS PARTICLES IN ONCORNAVIRUS CARRIER CELL LINES. III. SURVEY OF EFFECTS ON A-, B-, AND C-TYPE ONCORNAVIRUSES. (Eng.) Billiau, A. (Div. of Microbiology, Rega Inst., Univ. of Leuven, Leuven, Belgium); Edy, V. G.; De Clercq, E.; Heremans, H.; De Somer, P. *Int. J. Cancer* 15(6):947-953; 1975.

The effect of interferon on the synthesis and releas of A-, B-, and C-type viruses by oncornavirus carrie

lines was studied. Murine cell lines were selected which carry either of these viruses and are sensitive to the antiviral effect of interferon, as measured by inhibition of vesicular stomatitis virus. Cell monolayers were incubated with interferon at various concentrations. After 24 hr the medium was renewed, and interferon was again included. Tritiated uridine was added, and the cultures were further incubated for a 2- or 24-hr period. Medium was harvested for determination of B- or C-type virus release. Cells were thoroughly washed and extracted for determination of A-type particles. Release of C-type virus was highly sensitive. In two cell lines tested (MO-P and JLSV5) the concentration of interferon necessary to inhibit release of endogenous C-type virus was comparable to that necessay to inhibit exogenous VSV. In both cell lines, 100 or more reference units of interferon reduced radio-labeled C-type particles to an undetectable level. A concentration as high as 2,000 reference units/ml only marginally inhibited the release of B-type particles in L8A cells, which had otherwise good sensitivity to the anti-VSV effect of interferon; this indicates that B-type virus synthesis is poorly sensitive to interferon. Synthesis of intracisternal A-type particles was examined in interferon-sensitive MO-MVA cells; there was no significant difference between control cells and cells pre-treated with interferon. These results indicate that the release of noninfectious extracellular B-type virus, as well as the synthesis of noninfectious intracellular A-type particles, is markedly less sensitive to interferon than is the release of C-type viruses. This may indicate that interferon does not inhibit expression of an incorporated oncornavirus genome. It is also suggested that these differences may reflect fundamental differences in the synthesis of these viruses.

0822 PATHOGENESIS OF CYTOMEGALOVIRUS INFECTION. I. ACTIVATION OF VIRUS FROM BONE MARROW-DERIVED LYMPHOCYTES BY *IN VITRO* ALLOGENIC REACTION. (Eng.) Olding, L. B. (Scripps Clin. Res. Found., La Jolla, Calif.); Jensen, F. C.; Oldstone, M. B. A. *J. Exp. Med.* 141(3):561-572; 1975.

Pregnant and newborn C3H/HeJ, SWR/J, C3H/St, BALB/c, Ha/ICR, and nude mice were inoculated i.p. with 1,000 mean tissue culture infective doses (100 in newborns) of murine cytomegalovirus (MCMV) to determine if adult murine lymphocytes infected *in utero* and at birth carried MCMV and if MCMV could be activated *in vitro* by cocultivation with antigenically foreign tissue. Lymphoid cells were prepared from thymuses and spleens. Macrophages were removed by suspension in minimal essential medium with 5% heat-inactivated fetal calf serum through a nylon wool column. T-cells were purified by using rabbit antibody to B-cells in the presence of complement. B-cells were prepared in a similar manner. Suspensions of 1 x 10^6 to 5 x 10^6 cells from whole spleens, or purified B- or T- cells, were mixed with 1 x 10^5 to 2 x 10^5 murine cells from histoincompatible embryos and plated. Such suspensions from C3H/St mice infected at birth were cultured with lipopolysaccharide (10 μg/ml), concanavalin A (1, 5, and 10 μg/ml) or phytohemagglutinin-P (PHA-P, 50 μg/ml). After three days, 1.0 μCi (methyl-^{3}H) thymidine was added, and after four hrs, the cultures were chilled. The cells were counted in a scintillation chamber. MCMV was identified by immunofluorescence, antibody neutralization techniques, and electron microscopy. When spleen cells from C3H/HeJ or Ha/ICR mice were cocultivated with allogenic mouse embryo cells, cytopathic effects appeared, and MCMV was isolated in 83% of these cultures; MCMV was not isolated from controls. B-cell cultures resulted in isolation of MCMV in 66% *versus* 0% from T-cell cultures in the presence of allogenic mouse embryo cells. Pokeweed mitogen, concanavalin A, and PHA-P failed to activate MCMV, while lipopolysaccharide activated the virus. Thus, it is suggested that MCMV is harbored in B-cells and that the virus can be recovered from these cells when cocultivated with histoincompatible cells.

0823 CELLULAR COOPERATION IN TRANSFORMATION BY EB VIRUS. (Eng.) Furukawa, T. (Fox Chase Cancer Cent., Philadelphia, Pa.). *Res. Commun. Chem. Pathol. Pharmacol.* 10(3):554-558; 1975.

The Epstein-Barr virus-induced transformation of human blood leukocytes was investigated to determine the effects of removal of T-cells from other mononuclear leukocytes and addition of conditioned medium produced by mononuclear adhering cells. Removal of most of the T-cells facilitated transformation by Epstein-Barr virus. This could be caused by either an increased number of susceptible cells in the inoculum or an inhibitory effect of T-cells. After removal of the T-cells and the separation of adhering and nonadhering cells, neither adhering nor nonadhering cells were transformed. Recombining the cells restored susceptibility to Epstein-Barr virus transformation while addition of a 7-day conditioned medium from a culture of adhering mononuclear leukocytes to nonadherent cells did not. The cooperative effect of adhering and nonadhering cells on transformation previously reported for mononuclear leukocytes remained after removal of most T-cells. The role of adherent cells could not be substituted by conditioned medium. This is in contrast with previous studies with *in vitro* lymphocyte stimulation by antigens in which conditioned medium without cells was sufficient.

0824 ATTEMPTS TO DETECT VIRUS-SPECIFIC DNA SEQUENCES IN HUMAN TUMORS. III. EPSTEIN-BARR VIRAL DNA IN NON-LYMPHOID NASOPHARYNGEAL CARCINOMA CELLS. (Eng.) Wolf, H. (Institut für Klinische Virologie der Universität Erlangen-Nürnberg, Erlangen, West Germany); zur Hausen, H.; Klein, G.; Becker, V.; Henle, G.; Henle, W. *Med. Microbiol. Immunol. (Berl.)* 161(1):15-21; 1975.

Fourteen biopsies of nasopharyngeal carcinomas were analyzed for the presence of Epstein-Barr (EB) virus-specific DNA by DNA-complementary RNA hybridization. These data were compared with the histology of the respective tumors and the seroreactivity of the patients against EB virus-related antigens. For *in situ* hybridization experiments, frozen sections (10 μm) of biopsy material were fixed, covered by an

RNase solution (100 μg/ml in 2 x SCC), and kept for 30 min at 34 C. This was followed by denaturation of the section. Hybridization with EB virus-specific complementary RNA was performed with 50,000 cpm of complementary RNA in 50 μl of a solution containing 2.5 x SCC, 50% formamide, and 0.05% sodium dodecyl sulfate under a coverslip for 4 days at 45 C. The slides were then exposed to autoradiography. With one exception, all sections containing nasopharyngeal carcinoma cells hybridized significantly with EB virus-complementary RNA. The sera of all patients with tumors that hybridized with EB virus-DNA reacted at high titers against EB virus-specific antigens. *In situ* hybridization of frozen sections from a tumor containing nearly equal amounts of tumor cells and lymphocytes showed hybridizing DNA within the nuclei of the nonlymphoid cells. Although these data do not exclude the presence of EB virus-DNA within lymphoid cells, it is concluded that in nasopharyngeal carcinomas the vast majority of EB virus-specific DNA rests within the nonlymphoid cells.

0825 BIOCHEMICAL PROPERTIES OF A HAMSTER SYNCYTIUM-FORMING ("FOAMY") VIRUS. (Eng.)
Hruska, J. F. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.); Takemoto, K. K. *J. Natl. Cancer Inst.* 54(3):601-605; 1975.

The nucleic acid of hamster syncytium-forming "foamy" virus (HFV) was characterized. The HFV virus was isolated from simian virus 40 (SV40)-induced hamster embryo tumor cells (NHE) after cloning. HFV-infected cultures of NHE were labeled with either tritiated uridine (^{3}H-UDR) or tritiated thymidine (^{3}H-TDR) and a comparison of the trichloroacetic acid-precipitable counts were made. ^{3}H-UDR was incorporated into the 1.16 g/ml region where the virus banded; the ^{3}H-TDR was not incorporated. The ^{3}H-UDR peak was eliminated by NaOH. The nucleic acid of HFV was thus RNA. Actinomycin D-treated HFV-infected cells inhibited HFV growth, implying that DNA synthesis was required for HFV replication. An RNA-directed DNA polymerase was indicated in a reverse transcriptase activity assay. The HFV culture contained no C-type virus interspecies group specific antigen, showing that the reverse transcription activity was not due to contaminating C-type virus particles. On sucrose density gradients, the HFV yielded multiple size values with 62S 25-30%, 40S 5-10%, 18-20S 10-15%, and 4-7S 40-50% of the total RNA. The 18-20S and 20-30S peaks may represent host cell ribosomal RNA which is randomly enclosed within the virion as the virus buds from the cell surface. The 60-65S RNA peak represents all or part of the virus genome. Because of the above characteristics, the HFV may be classified in a separate subgroup of the oncornaviruses.

0826 MODIFICATIONS OF CELLULAR RNA-POLYMERASE II AFTER INFECTION WITH FROG VIRUS 3. (Eng.)
Campadelli-Fiume, G. (Instituto di Microbiologia, Università di Bologna, Via San Giacomo, 12, I-40126 Bologna, Italy); Costanzo, F.; Foa'-Tomasi, L.; La Placa, M. *J. Gen. Virol.* 27(3):391-394; 1975.

RNA polymerase II extracted from frog virus 3 (FV3)-infected and uninfected BHK cells were compared for their ability to bind [^{3}H]-amanitin and ribonucleoside triphosphates. Experiments were carried out on BHK cell monolayers infected with FV3 at an input multiplicity of 10-20 plaque-forming U/cell and incubated at 26 C. Amanitin binding sites and dissociation constants of the complexes formed between amanitin and RNA polymerase II were determined 4 and 12 hr postinfection by equilibrium dialysis experiments. Both the binding sites and the dissociation constants were significantly modified following FV3 infection. At 4 and 12 hr postinfection, respectively, the number of amanitin binding sites in RNA polymerase II was 1/2 and 1/3 of that in RNA polymerase II extracted from uninfected cells. This decrease in amanitin binding sites paralleled impairment of RNA-polymerase II activity in infected cells, as demonstrated by reduced incorporation of uridine monophosphate. The dissociation constant of the complex between amanitin and RNA polymerase II extracted from 4-hr infected cells was 6.2×10^{-9} M, compared with 2.9×10^{-9} M for uninfected cells. At 12 hr after infection, the value of the dissociation constant was 7.7×10^{-9} M. Michaelis-Menton constants of RNA polymerase II for ribonucleoside triphosphates remained unchanged following FV3 infection. This indicates that, in infected cells, the fraction of enzyme molecules still capable of catalyzing RNA synthesis retain a normal affinity for ribonucleotides.

0827 VIRAL CARCINOGENESIS IN A PRONEPHRIC CELL LINE: AN ULTRASTRUCTURAL STUDY. (Eng.)
Wong, W. Y. (Dept. of Biology, Univ. of Notre Dame, Notre Dame, Indiana); Tweedell, K. S. *Am. J. Pathol.* 80(1):143-152; 1975.

Herpesvirus recovered from cell fractions of the spontaneous Lucké renal tumor of the adult frog (*Rana pipiens*) were used to infect a cell line derived from pronephroi of the same species. The cytopathology of the infected frog pronephric cell and the frog herpesvirus development were studied with electron microscopy. The frog pronephric cells were cultivated at 25 C in Leibovitz medium. The oncogenic herpesvirus was recovered from cell fractions of a spontaneous frog kidney tumor by homogenization and differential centrifugation. Infected pronephric cells were sedimented at 800 x *g*. The cell pellet was fixed for 1-2 hr at 4 C in 4% glutaraldehyde made up in 0.1 M $NaPO_4$ buffer. It was infiltered in a propylene oxide-Epon (1:1) mixture and embedded in Epon 812. Thin sections were double stained with uranyl acetate and lead citrate. Viruses and virus-associated structures previously found in the primary renal tumor were observed, including nuclear inclusions of capsids with single or double membranes and capsids with nucleoids often within nuclear sacs. Embedded within the clumped and marginated chromatin were 55-nm tubular elements and associated unit membrane structures. Virus-associated, 35-nm tubular elements were also seen. The cytoplasm contained single, enveloped nucleoid virus and clusters of virus within cytoplasmic vesicles. Other cytoplasmic inclusions were dense, virus-associated 25-nm filaments; virus particles within myeloid bodies; and possible viral budding from tubular organelles. The

development of the frog herpesvirus in the pronephric cells showed strong similarities to the development of *Herpes simplex* virus, to herpes B virus, and to infectious bovine rhinetracheitis virus. It is suggested that this development parallels the developmental events of the herpesvirus in the Lucké tumor.

0828 INFECTION OF CAPUCHIN MONKEYS (*CEBUS ALBIFRONS*) WITH *HERPESVIRUS SAIMIRI*. (Eng.) Rabin, H. (Litton Bionetics, Inc., Kensington, Md.); Pearson, G. R.; Wallen, W. C.; Neubauer, R. H.; Cicmanec, J. L.; Orr, T. W. *J. Natl. Cancer Inst.* 54(3):673-677; 1975.

A chronic, nononcogenic infection by *herpesvirus saimiri* (HVS) in capuchin monkeys (*Cebus albifrons*) is described. Five adult capuchin monkeys were inoculated with HVS. All infected monkeys seroconverted to HVS-associated early antigen (EA) and late antigen (LA). After three months, antibody to LA reached titers of 1:40-1:160. During the observation period, no prolonged changes in mitogen response pattern to phytohemagglutinin (PHA) or to pokeweed mitogen (PWM) were noted. Stimulation indexes were generally consistent with mitogen used. No persistent signs of clinical diseases were found in physical or hematologic examination. Two specimens did show transitory lymphadenopathy. Peripheral lymphocyte counts remained low (1,600-11,500 cells/mm^3). Prednisolone treatment was found to give an immunosuppressive effect with only transitory response in peripheral blood lymphocyte level and mitogen response. Cellular immunity to virus antigen, in chronically infected animals, could have been responsible for the inhibition of lymphoma production.

0829 CHROMOSOME ABERRATIONS IN SYRIAN HAMSTER EMBRYO CELLS TRANSFORMED AFTER EXPOSURE TO ULTRAVIOLET-IRRADIATED HERPES SIMPLEX VIRUS TYPE 1 OR 2. (Eng.) Nachtigal, M. (Univ. Craiova, Rumania); Duff, R.; Rapp, F. *J. Natl. Cancer Inst.* 54(1):97-105; 1975.

Chromosomal aberrations were analyzed in Syrian hamster cell lines FR-6-1, MS-4-1, 333-2-29, 333-8-9, KOS-6-1 and 14-012-8-1 following exposure of cultures to UV-irradiated herpes simplex virus (HSV) type 1 or 2 to characterize chromosomal aberration patterns of the HSV-transformed cells. The HSV-transformed cell lines showed chromosome stability. They also demonstrated a low incidence of endoreduplications, polyploids and metaphases with damaged chromosomes. The cell lines were passaged more than 100 times and developed marker chromosomes, suggesting clonal type evolution. Negatively stained chromosomal regions were frequently found in all HSV-transformed lines. Two structurally different marker chromosomes were found in the 333-8-9 and 14-012-8-1 lines. The negatively stained regions in all of the cell lines except 333-8-9 and 14-012-8-1 appeared to be secondary constrictions. The heterochromatic regions of the 333-8-9 and 14-012-8-1 lines suggested the presence of an unstained ovoid body. A high incidence of chromosomal breakage was seen only in the highly tumorigenic 333-2-29 cell line. It is suggested that the abnormal heterochromatic regions of the marker chromosomes may have been due to HSV. The chromosomal stability is suggested to be due to the weakening of HSV by ultraviolet radiation.

0830 SELECTIVE INHIBITION OF HERPES SIMPLEX VIRUS BY 5'-AMINO-2',5'-DIDEOXY-5-IODOURIDINE. (Eng.) Cheng, Y. C. (Dep. Pharmacol., Yale Univ., New Haven, Conn.); Goz, B.; Neenan, J. P.; Ward, D. C.; Prusoff, W. H. *J. Virol.* 15(5): 1284-1285; 1975.

The antiviral and cytotoxic properties of the thymidine analog, 5'-amino-2',5'-dideoxy-5-iodouridine (AIU) are described and compared to other antiviral agents. Vero cells were infected with herpes simplex virus type 1 (HSV-1) at a multiplicity of infection of 10. AIU was found to be less potent than 5-iodo-2'-deoxyuridine (IdUrd), fluoromethyl-2'-deoxyuridine (F_3dThD) and cytosine arabinoside (ara-C) but, on a molecular basis, AIU possessed greater activity than adenosine arabinoside (ara-A). The cytotoxicity of AIU was negligible with no growth inhibition in concentrations up to 200 μM. No detectable influence was noticed on morphological characteristics or on the rate of DNA and RNA synthesis. The presence of the 5' amino group of AIU may account for the lack of its cytotoxicity. AIU affects an intracellular event in virus replication and inhibits the uptake of ^{14}C-thymidine into DNA.

0831 DIFFERENTIAL ACTION OF DEOXYNUCLEOSIDES ON MAMMALIAN CELL CULTURES INFECTED WITH HERPES SIMPLEX VIRUS TYPES 1 AND 2. (Eng.) Kelman, A. D. (Boston Univ. Sch. Med., Mass.); Capozza, F. E.; Kibrick, S. *J. Infect. Dis.* 131(4):452-455; 1975.

The effects of thymidine (5 mM) on the cytopathic effects (CPE) induced in human amnion cultures of eight strains of Herpes Simplex virus (HSV) types 1 and 2 were determined. Cultures were examined at intervals of approximately 12 hr. Thymidine at a 5 mM concentration was not toxic to the human amnion monolayers. In each case, CPE caused by HSV-2 was markedly inhibited by thymidine. In contrast, this deoxynucleoside had no significant inhibitory effect on cultures infected with strains of HSV-1. At concentrations up to 1 mM thymidine, deoxycytidine and deoxyguanosine delayed CPE in rabbit kidney cells infected with HSV-2. A combination of the two pyrimidines gave results similar to those with thymidine alone. CPE in cells infected with HSV-2 was suppressed longer in cultures treated with thymidine. This effect was maximal at 48 hr. At 72 hr the thymidine treated cultures had progressed to 100% CPE, but those with deoxycytidine had developed only 50% CPE. Deoxyguanosine may be a more powerful inhibitor of HSV-2 CPE in rabbit kidney cells. Only minute foci did not progress, even after seven days. The data suggest that excess thymidine in the culture media provides a simple method for discriminating strains of HSV-1 from those of HSV-2.

0832 RNA SUBUNIT STRUCTURE OF MASON-PFIZER MONKEY VIRUS. (Eng.) Schochetman, G. (Meloy Lab., Springfield, Va.); Schlom, J. *J. Virol.* 15(2):423-427; 1975.

The 60-70S RNA subunit structure of Mason-Pfizer monkey virus was determined and compared with that of Rauscher murine leukemia virus. The Mason-Pfizer monkey virus 60-70S RNA had a molecular weight of 8 x 10^6 when analyzed on polyacrylamide gels. Dissociation of 60-70S RNA of Mason-Pfizer monkey virus and murine leukemia virus by heat or formamide (40%) resulted in conversion to identical subunit structures of 2.8 x 10^6 daltons. Treatment with lower amounts of formamide revealed a partial dissociation to intermediate RNA structures. Complete heat dissociation of Mason-Pfizer monkey virus 60-70S RNA released three low molecular weight RNA species of 10^5, 3.5 x 10^4, and 2.5 x 10^4. These molecular weights were calculated to correspond to approximate sedimentation coefficients of 4S, 4.5S, and 7S, resp. They were similar to those found in Rous sarcoma virus. The 4S, 4.5S, and 7S RNAs were present in counts/min ratios of 8:3:4 and therefore represent approximately 4, 1.5 and 2%, resp., of the total counts/min of the original 60-70S RNA molecule. The stepwise dissociation of Mason-Pfizer monkey virus 60-70S RNA by limited formamide treatment suggests that the RNA subunits of the virus may be linked by differentially stable hydrogen bonded regions. Similar results were obtained in other studies with the 60-70S RNA of avian myeloblastosis virus. This investigation demonstrates the similarities of Mason-Pfizer monkey virus RNA in size, subunit, structure and associated RNAs to those of known avian and mammalian RNA tumor viruses.

0833 IMMUNOLOGICAL MECHANISMS IN THE PATHOGENESIS OF VIRUS-INDUCED MURINE LEUKEMIA. I. AUTOREACTIVITY. (Eng.) Proffitt, M. R. (Massachusetts Gen. Hosp., Boston); Hirsch, M. S.; Gheridian, B.; McKenzie, I. F. C.; Black, P. H. *Int. J. Cancer* 15(2):221-229; 1975.

Thymocyte suspensions were prepared from three or four pooled thymuses from normal or leukemia virus-carrying C3H/HeJ mice and were tested using a cytotoxicity assay to see if viruses might directly trigger proliferation of autoreactive lymphoid cell clones. Leukemia was induced in C3H/HeJ mice by injections of Moloney murine leukemia virus (MuLV-M, LTV strain). Subsequent generations transmitted the virus to their offspring causing an 90-100% incidence of leukemia in these carriers. Two sublines (one infected with MuLV-M, and one uninfected) of C3H/HeJ embryo cell cultures were also established. Thymocytes from MuLV-M-carriers decreased numbers of normal, uninfected syngeneic target cells but did not decrease numbers of MuLV-M-infected target cells. The same pattern of reactivity was seen during both pre- and postneoplastic periods. Normal mouse thymocytes slightly stimulated infected and uninfected target cell replication. Lymphoma cells obtained from a mouse with overt thymic lymphoma demonstrated similar cytotoxicity. Spleen and lymph node lymphocytes from preleukemic young (8 to 12 weeks old) carriers reacted against MuLV-M-infected target cells, but not against non-infected ones. Lymphocytes from older (16 to 17 weeks old) preleukemic carriers reacted like MuLV-M-carrier thymocytes. The authors suggest that an autoreactive process of thymic origin may play a role in MuLV-M-induced thymic lymphoma.

0834 IMMUNOLOGICAL MECHANISMS IN THE PATHOGENESIS OF VIRUS-INDUCED MURINE LEUKEMIA. II. CHARACTERIZATION OF AUTOREACTIVE THYMOCYTES. (Eng.) Proffitt, M. R. (Massachusetts Gen. Hosp., Boston); Hirsch, M. S.; McKenzie, I. F. C.; Gheridian, B.; Black, P. H. *Int. J. Cancer* 15(2):230-240; 1975.

The reactivity of thymocytes from Moloney murine leukemia virus (MuLV-M)-infected mice against uninfected normal syngeneic thymocytes was found to be mediated by viable murine leukemia virus-infected thymocytes. Antisera against C58 strain mouse thymus cells, mouse IgG, B_{10}BR mouse thymus lymph node and spleen, and MuLV-M-induced tumor cells were prepared to test for alloantigens. Using a microcytotoxicity assay, lysates of MuLV-M-carrier thymocytes and normal cells were similar in reducing numbers of MuLV-M-infected and uninfected target cells. The thymocytes involved in the reaction were found to be positive for the H-2^k and theta-C3H alloantigens and were negative for IgG determinants. Carrier and normal mice were treated with 2.5 mg/20 g of hydrocortisone, and thymocyte suspensions were prepared 48 hours later. Thymocytes from the MuLV-M-carriers were not affected by the hydrocortisone treatment, indicating that they were functionally mature immunocytes.

0835 TUMOR INDUCTION BY MOLONEY SARCOMA VIRUS IN ATHYMIC NUDE MICE. (Eng.) De Clercq, E. (Rega Inst. Med. Res., Univ. Leuven, Belgium). *J. Natl. Cancer Inst.* 54(2):473-477; 1975.

Moloney murine sarcoma virus (M-MSV)-induced tumor formation was assessed in athymic nude (nu/nu) mice and compared with tumor development in normal NMRI (+/+) mice and nu/+ heterozygotes. The mice were infected i.m. with M-MSV at either 2 or 30 days of age. Newborn mice received 0.05 ml of a $10^{-2.0}$ dilution of M-MSV; adult mice were given 0.1 ml of a $10^{-1.0}$ virus dilution. Athymic nude mice, normal NMRI mice, and the heterozygotes inoculated at birth with M-MSV did not differ significantly in the latency period to tumor appearance, though the rate of tumor appearance and final tumor incidence were slightly lower in the nu/nu group than in the nu/+ and +/+ groups. The mortality rate was somewhat higher in the nu/nu group than in the other two groups. Athymic nude and normal NMRI mice inoculated at 30 days of age with M-MSV did not differ significantly in the latency period to tumor appearance, rate of tumor appearance, or tumor incidence. However, tumors induced in adult normal NMRI mice invariably regressed, whereas all tumors induced in adult nude mice continued to grow until they killed the hosts. In the +/+ group, all mice survived; in the nu/nu group, all mice succumbed.

These results indicate that thymus-dependent immunity, though not active in M-MSV tumor induction, determines whether the tumor, once formed, will regress or kill the host.

0836 ONCORNAVIRAL PROTEIN MODULATION IN MOUSE UTERINE TISSUE BY ESTROGEN. (Eng.)
Fowler, A. K. (Natl. Cancer Inst., Bethesda, Md.); Kouttab, N. M.; Kind, P. D.; Strickland, J. E.; Hellman, A. *Proc. Soc. Exp. Biol. Med.* 148(1):14-18; 1975.

The time sequence of the appearance of group-specific (gs) protein and an RNA directed DNA polymerase in uterine tissue of ovariectomized NIH-Swiss mice following a single estrogen treatment of near physiological dosage was determined. The level of the murine leukemia virus gs protein and the activity of an RNA-directed DNA polymerase in the uterus was elevated by treatment with estrogens. The extent that these markers were raised was dependent on the relative biological potency of the estrogen and on the time interval following treatment. Estradiol-17β ($E_2β$), the more potent estrogen, was the most effective activator of gs protein. In mice receiving as little as 0.01 μg $E_2β$, gs protein was detectable after 24 hr. By comparison 1.0 μg estrone or estriol was required to induce detectable levels of gs protein at 24 hr, although a lesser amount (0.01 μg) was effective by 72 hr. Estradiol-17α, the least active estrogen examined, was the weakest inducer of gs protein. By this method of assay, gs protein was not detected in the uteri of sham-treated mice or of mice receiving 0.001 μg of the estrogens. Increases in the levels of both viral marker proteins were evident within 24 hr of treatment and were highest at 48 hr. Subsequently, viral protein levels declined to pretreatment levels. The results provide additional evidence demonstrating hormonal influence of uterine virogene marker expression. This is supported by the observations that the efficacy of several natural estrogen metabolites to activate these markers is associated with their relative biological potency and, further, that the kinetics of the estrogen initiated response follows a highly predictable temporal sequence.

0837 INHIBITION OF ONCORNAVIRUS FUNCTION BY 2'-AZIDO POLYNUCLEOTIDES. (Eng.) De Clercq, E. (Rega Inst. Medican Res., Univ. Leuven, Belgium); Billiau, A.; Hobbs, J.; Torrence, P. F.; Witkop, B. *Proc. Natl. Acad. Sci. USA* 72(1):284-288; 1975.

The anti-oncornavirus activity of poly(2'-azido-2'-deoxyuridylic acid) [poly(dUz)] and poly(2'-azido-2'-deoxycytidylic acid) [poly(dCz)] is described. Both poly(dUz) and poly(dCz) were found to inhibit the RNA-directed DNA polymerase (reverse transcriptase) activity of the Moloney and Rauscher strains of murine leukemia virus, Moloney murine sarcoma virus, Theilen feline leukemia virus, and Gardner feline sarcoma virus. Under the same conditions, the unsubstituted parent compounds failed to inhibit reverse transcriptase. Poly(dUz) and poly(dCz) inhibited the replication of exogenous Moloney strain murine sarcoma virus in nontransformed MO cells, but poly(dUz) failed to suppress the formation of endogenous sarcoma and leukemia viruses in transformed MO-P and JLSV5 cell lines. In these same cells, poly(dUz) did not inhibit the multiplication of vesicular stomatitis virus. It is suggested that reverse transcriptase is necessary for the productive infection and transformation of normal cells by oncornaviruses but is not essential for the maintenance of the transformed state and the continuous production of new virus particles by these transformed cells.

0838 QUALITATIVE AND QUANTITATIVE INTERACTIONS OF LECTINS WITH UNTREATED AND NEURAMINIDASE-TREATED NORMAL, WILD-TYPE, AND TEMPERATURE-SENSITIVE POLYOMA-TRANSFORMED FIBROBLASTS. (Eng.) Nicolson, G. L. (Salk Inst. Biological Studies, San Diego, Calif. 92112); Lacorbiere, M.; Eckhart, W. *Biochemistry* 14(1):172-179; 1975.

The lectin receptors of confluently grown baby hamster kidney (BHK), wild-type polyoma virus transformed PyBHK, and temperature-sensitive polyoma transformed ts3-PyBHK fibroblasts were studied using cell agglutination, quantitative (^{125}I)lectin binding and ferritin-lectin labeling. PyBHK and permissively grown ts3-PyBHK cells agglutinated more strongly with *Ricinus communis* I agglutinin (RCA_I) compared with BHK and nonpermissively grown ts3-PyBHK cells. Saturation binding of (^{125}I)RCA_I to these cells at 4 C resulted in a 2-fold difference in lectin-binding sites on BHK and nonpermissively grown ts3-PyBHK cells (1.0-1.3 x 10^7 sites/cell) compared with PyBHK and permissively grown ts3-PyBHK (0.4-0.6 x 10^7 sites/cell). These cells bound equivalent amounts of (^{125}I)concanavalin A (0.8-1.0 x 10^7 sites/cell) and (^{125}I)wheat germ agglutinin (1.0-2.2 x 10^7 sites/cell). Under these conditions, little endocytosis occurred, as judged by the subsequent release of more than 90% cell-bound (^{125}I)RCA_I by the RCA_I inhibitor lactose and localization of ferritin-RCA_I exclusively to the extracellular plasma membrane surface. If the binding is performed at 22 C, only 50% of the bound lectin can be removed by lactose, and ferritin-RCA_I is localized inside the cell within endocytotic vesicles. The relative mobility of RCA_I receptors was examined on ts3-PyBHK cells by the ability of ferritin-RCA_I to induce clustering of its receptors at 22 C. RCA_I receptors on permissively grown cells appeared to be more mobile than on nonpermissively grown ts3-PyBHK cells. BHK and PyBHK cells were treated with neuraminidase, and the resulting cells were assayed for lectin agglutinability and quantitative binding of RCA_I, concanavalin A, and wheat germ agglutinin. Neuraminidase treatment resulted in decreased concanavalin A and wheat germ agglutinability and a slight increase in RCA_I agglutinability. The enzyme-treated BHK and PyBHK boun less (^{125}I)wheat germ agglutinin (2.8 x 10^6 and 2.2 x 10^6 sites/cell, resp.) and 2.5 and 6.3 times more (^{125}I)RCA_I (2.5-3.0 x 10^7 and 3.5-4.0 x 10^7 sites/cel resp.). There was no change in the number of concanavalin A binding sites.

0839 POLYOMA VIRUS T ANTIGEN. I. SYNTHESIS OF MODIFIED HEAT-LABILE T ANTIGEN IN CELLS TRANSFORMED WITH THE ts-a MUTANT. (Eng.) Paulin, D. (Dep. Mol. Biol., Inst. Pasteur, Paris, France); Cuzin, F. *J. Virol.* 15(2):393-397; 1975.

The synthesis of T-antigen in mouse 3T3 cells transformed either by wild type (PY-3T3 PY-6) or by a temperature-sensitive mutant of polyoma (ts-a-3T3) virus was investigated to determine if T-antigen is the product of a viral gene. Cells were grown on cover slips at several temperatures, fixed, and stained by the indirect immunofluorescence technique, using polyoma anti-T hamster serum and fluorescein-coupled anti-hamster immunoglobulin (goat) antiserum. In PY-6 cells grown at 39 C or shifted for various periods of time up to 48 hr at 31 C, a typical immunofluorescence staining was observed in more than 80% of the cells. Correspondingly high antigen concentrations were measured in extracts by complement fixation assays. In mouse cells transformed with ts-a-3T3, only low amounts (1 in 1000 cells) of the virus specific T-antigen were synthesized at high temperature (39 C). After a shift-down to the permissive temperature (31 C), these cells exhibited the same level of T-antigen production as wild type polyoma transformants. The T-antigen produced by ts-a-transformed cells was inactivated at 39 C *in vitro* at a faster rate than that produced by wild type transformed cells. Loss of T-antigenic activity in extracts at 39 C, correlated with the ts genotype of the virus and the absence of detectable T antigen in 95% of cells growing at this temperature, strongly suggests that the product of the viral gene carries the T-antigenic site.

0840 *IN VITRO* POLYOMA DNA SYNTHESIS: REQUIREMENT FOR CYTOPLASMIC FACTORS. (Eng.) Francke, B. (Tumor Lab., Salk Inst., San Diego, Calif. 92112); Hunter, T. *J. Virol.* 15(1):97-107; 1975.

DNA synthesis was studied in unfractionated lysate and purified nuclei (in the absence and presence of cytosol) from BALB mouse 3T3 cells infected with a temperature-sensitive polyoma virus mutant (ts 1260). Lysates were prepared by treating the infected cells with hypotonic buffer and mixing lysate and sucrose buffer in a 9:1 ratio. Nuclei were prepared from the lysate by dilution in hypotonic buffer, followed by centrifugation with 20% Ficoll in isotonic buffer. Cytosol was prepared by homogenizing the hypotonic lysate, followed by high-speed centrifugation (30,000 x *g*). The supernatant was used as the cytosol fraction. For DNA characterization, the Hirt supernatant fraction was sedimented through a neutral sucrose gradient. After concentration, the DNA was further analyzed by sedimentation through alkaline sucrose gradients. Purified nuclei from infected cells were greatly reduced in their ability to synthesize viral DNA *in vitro* when compared with unfractionated hypotonic lysate of the infected cells. The synthetic capacity of the nuclei was fully reconstituted upon addition of cytosol from infected or uninfected cells. Optimal complementation required high concentrations of the cytosol. Most of the complementing activity was heat labile, being destroyed at 60 C. Dialysis had no effect on the activity. Analysis of the viral DNA synthesized in purified nuclei showed an accumulation of Okazaki-type short DNA chains, which could be chased into viral progeny DNA strands if cytosol was added back to the nuclei. Kinetic analysis of the pulse-labeling pattern of viral replicative DNA showed a strong dependence of the extension of viral progeny strands and of the processing of Okazaki-type fragments on the amount of cytosol present during the reaction. It is suggested that the cytoplasmic DNA polymerase may be one of the active components in the cytosol.

0841 STUDIES OF POLYOMA VIRUS DNA: CLEAVAGE MAP OF THE POLYOMA VIRUS GENOME. (Eng.) Chen, M. C. Y. (Natl. Inst. Allergy and Infectious Diseases, Bethesda, Md. 20014); Chang, K. S. S.; Salzman, N. P. *J. Virol.* 15(1):191-198; 1975.

Cleavage of a newly isolated strain of polyoma virus, MPC-1, was effected by endonuclease R *Haemophilus influenzae* d restriction enzymes (*Hin* d). A physical map of the polyoma virus genome was constructed. Mouse embryo cultures were infected with MPC-1 at a multiplicity of infection of about 1 plaque-forming U/cell. $(^{32}P)P_1$ (50 µCi/ml) was added to the infected cells 24 hr postinfection. At 68 hr postinfection, low molecular wt DNA was extracted with sodium dodecyl sulfate by the Hirt method. ^{32}P-labeled polyoma virus DNA was also extracted from virions purified by sedimentation onto a CsCl cushion. Labeled virus was digested with 0.03 U of endonuclease R *Hin* d in 0.05 ml of 50 mM NaCl-6.6 mM Tris hydrochloride-6.6 mM $MgCl_2$ at 37 C for 16 hr. The reaction was stopped by the addition of 0.02 M EDTA. To obtain incomplete digests of DNA, the ratio of enzyme to DNA and the time of incubation were varied. Cleaved DNA was analyzed by electrophoresis on 3% polyacrylamide-0.05% agarose gels and by electron microscopy. After complete digestion of MPC-1 DNA by *Hin* d, four distinct DNA fragments were resolved by electrophoresis. They correspond to fragments of 18.2, 7.9, 2.8, and 2.4 x 10^{-5} molecular weight. Length measurements of electron micrographs agree with these values.

0842 TANDEM REPETITION OF THE ORIGIN OF DNA REPLICATION IN DEFECTIVE POLYOMA VIRUS DNA'S. *(Eng.)* Folk, W. R. (Dep. Biol. Chem., Univ. Michigan, Ann Arbor); Fishel, B. R. *Virology* 64(2):447-453; 1975.

The sequences in a population of noninfectious covalently closed circular DNAs derived from polyoma virus were characterized. Specific fragments of polyoma DNA produced by cleavage with endonuclease *Hpa*II from *Haemophilus parainfluenzae* were separated by polyacrylamide-gel electrophoresis, and the kinetics of reassociation of the *Hpa*II fragments of polyoma ^{32}P-labeled DNA were measured in the presence and absence of an excess of *Eco*R_1-resistant ^{3}H-labeled DNA fragments. Infectious polyoma DNA was cleaved by *Hpa*II into eight fragments; band 2 on the polyacrylamide gels used to separate these fragments contained the single site in the polyoma

genome which is cleaved by the *Eco*R$_1$ endonuclease. Those DNAs lacking the *Eco*R$_1$ site were not infectious. *Hpa*II endonuclease digestion of the noninfectious DNAs produced a single major fragment which was identified as *Hpa*II-E. *Hpa*II digestion of the *Eco*R$_1$-sensitive DNAs derived from the same infection as the *Eco*R$_1$-resistant DNAs generated eight major fragments with electrophoretic mobilities similar or identical to those of fragments produced from infectious, low multiplicity polyoma DNA. High multiplicity polyoma virus infection produced a reassortment of sequences within the polyoma genome, generating regions of DNA which had a smaller or greater number of *Hpa*II sites than is normally found. The majority of the repeated sequences in the noninfectious DNA were complementary to fragment E and parts of fragments C and D of the polyoma genome; it has previously been shown that the origin of DNA replication is located at the boundary of fragments E and C. Serial repetition of the origin of repetition may have occurred even in molecules containing the *Eco*R$_1$ site.

0843 EFFECT OF UV LIGHT ON RNA-DIRECTED DNA POLYMERASE ACTIVITY OF MURINE ONCORNAVIRUSES. (Eng.) Lovinger, G. G. (Flow Lab., Inc., Rockville, Md.); Ling, H. P.; Gilden, R. V.; Hatanaka, M. *J. Virol.* 15(5):1273-1275; 1975.

The susceptibility of endogenous DNA polymerase of Rauscher leukemia virus to UV irradiation at 254 nm with 40 ergs per mm^2 was investigated. The infectivity titer measured by the XC plaque assay dropped rapidly with a 37% survival time in less than one min of irradiation. The major internal viral protein, p30, showed great resistance to UV inactivation. The UV inactivation rates for endogenous and exogenous viral polymerases indicated single-hit kinetics with a 37% surviving activity reached by 10-13 min. The inactivation rates of both activities were similar; unirradiated nucleic acid template and primer, when added in the exogenous reaction, did not reverse inactivation. The rate of loss of activity at 254 nm was about 65% the rate of loss at 280 nm. At least 5-10% of the loss of infectivity of irradiated preparations is thought to be due to viral polymerase inactivity, because this enzyme is essential for oncornavirus infectivity.

0844 FATE OF VIRAL RNA OF MURINE LEUKEMIA VIRUS AFTER INFECTION. (Eng.) Takano, T. (Natl. Inst. Arthritis, Metab., Dig. Dis., Bethesda, Md.); Hatanaka, M. *Proc. Natl. Acad. Sci. USA* 72(1):343-347; 1975.

The reverse transcription of viral RNA into DNA was studied. (^{3}H)uridine-labeled Rauscher leukemia virus was used to infect mouse embryo fibroblasts of the BALB/c strain. The infected cells were separated into nuclear and cytoplasmic fractions by treatment with a 1% solution of neutral detergent NP-40 in 0.14 M NaCl, 0.02 M $MgCl_2$, and 0.02 M Tris x HCl, pH 7.2. Nucleic acid was extracted by sodium dodecyl sulfate-phenol-chloroform treatment and analyzed by Cs_2SO_4 and sucrose density gradient centrifugation. Between 45 and 70 min after infection, a transient and synchronized shift of the acid-insoluble radioactive peak toward the RNA-DNA hybrid region occurred in both the nuclear and cytoplasmic fractions. The density of the cytoplasmic hybrid shifted to 1.56 g/ml (RNA = about 50%), while the sedimentation rate decreased from 36 S to 14 S. The density of the nuclear hybrid shifted from 1.58 to 1.48 g/ml (RNA = 57-17%, resp.), while its sedimentation rate remained about 65 S. Hybrids in the nuclear and in the cytoplasmic fractions still showed hybrid density after heat denaturation. It is suggested that the viral RNA-dependent DNA polymerase (reverse transcriptase) and/or a similar enzyme of the host cell is involved in the formation processes of these hybrid molecules. A possible integration of the viral genetic information into the host chromosomes is discussed.

0845 DEOXYRIBONUCLEIC ACID-DEPENDENT RIBONUCLEIC ACID POLYMERASES FROM SPLEEN OF UNINFECTED AND RAUSCHER MURINE LEUKEMIA VIRUS-INFECTED NIH SWISS MICE. (Eng.) Sethi, V. S. (Bionetics Res. Lab., Kensington, Md.); Gallo, R. C. *Biochim. Biophys. Acta* 269(2):269-281; 1975.

The RNA polymerase activities from the nuclei of spleens of uninfected and Rauscher murine leukemia virus-infected NIH Swiss mice were resolved by DEAE-cellulose column chromatography, and their properties were compared. RNA polymerase activities were eluted from spleen nuclei of leukemic and normal mice by low and high salt extraction. The majority of the enzyme activities was precipitated with 50% ammonium sulfate; for complete isolation of detectable RNA polymerase activities, 75% ammonium sulfate saturation was used. The enzymes from the nuclear extracts, obtained from both methods, were applied to DEAE-cellulose columns and the activities were analyzed with single-stranded and double-stranded calf thymus DNA and with poly[d(A-T)] as templates. Three major peaks of enzyme activity eluting at 0.09, 0.18, and 0.23 M ammonium sulfate were obtained, which were termed RNA polymerase I, II and III, resp. The RNA polymerase activities from infected and uninfected spleens were the same with respect to column elution profiles, optimum requirements for various salts, ratios of activities of Mn^{++} and Mg^{++}, sedimentation values, and response to most templates. With the exception of minor differences in activities with certain DNA templates, the significance of which is not clear, no qualitative differences in the enzymes were found. However, an increase in the specific activity of the α-amanitin sensitive enzyme, RNA polymerase II, was found in the leukemic spleen. These preliminary results suggest that no novel RNA polymerase is induced by Rauscher murine leukemia virus-infection, and they are in keeping with the interpretation that the viral genome is transcribed by a host RNA polymerase.

0846 THE METHIONYL TRANSFER RNAs OF ROUS SARCOMA VIRUS. (Eng.) Faras, A. J. (Univ. Michigan Med. Sch., Ann Arbor). *Virology* 63(2):583-588; 1975.

The methionyl RNAs of avian sarcoma viruses were

compared with those from chick embryo fibroblasts in an effort to detect differences and/or similarities between a transforming oncornavirus and its host cell. Rous sarcoma virus (RSV) and chick embryo fibroblast transfer RNA, aminoacylated with ^{35}S-labeled methionine, were applied to a 0.9 x 25 cm column of RPC-5. The isoaccepting species were eluted from the column. Fractions were precipitated and counted in a liquid scintillation spectrometer. The methionyl-containing transfer RNAs from chick embryo fibroblasts were resolved into four major peaks. Similarly, all four isoaccepting methionyl transfer RNA species were identified in virions of RSV, both free and associated with the 70 S RNA. When the methionyl transfer RNAs of RSV were chromatographed on benzoylated DEAE-cellulose, both the 70 S-associated and free methionyl transfer RNAs were resolved into two discrete peaks, suggesting that RSV contains both methionyl and *N*-formyl methionyl transfer RNA species. When the four RPC-5 and the two benzoylated DEAE-cellulose peaks were subjected to transformylation, only peak I from either RPC-5 or benzoylated DEAE-cellulose could be formulated by *E. coli* transformylase. It is concluded that virions of RSV contain the two major classes of methionyl transfer RNA, transfer RNA^{met} and transfer RNA$_f^{met}$, found in their host cells. It appears that more transfer RNA$_f^{met}$ is found associated with 70 S RNA than free in virions of RSV, suggesting a preferential association of the formylated methionyl transfer RNA species with the viral genome. Whether these methionyl transfer RNAs, selectively associated with the RSV genome, serve any function in the maintenance of the 70 S RNA complex or have any specific role in the life cycle of the avian RNA tumor viruses, remains to be determined.

0847 METABOLISM IN NORMAL AND VIRUS-TRANSFORMED CHICK EMBRYO FIBROBLASTS AS OBSERVED WITH GLUCOSE LABELED WITH ^{14}C AND TRITIUM AND WITH TRITIUM-LABELED WATER. (Eng.) Rambeck, W. A. (Lehrstul fuer Organische Chemie Biochemie, Technische Universitat, 8 Munchen 2, Arcisstr. 21, West Germany); Bissel, M. J.; Bassham, J. A. *Hoppe Seylers Z. Physiol. Chem.* 356(2):203-212; 1975.

Glucose metabolism in normal and virus-transformed chick embryo fibroblasts in culture was observed by allowing the cells to metabolize (U-^{14}C)glucose plus glucose labeled with tritium in the C-1, C-3, and C-6 positions. Similarities and differences between normal and transformed cells were observed and measured. Primary cultures were prepared from 10-day old C/O or C/B type chick embryos. For studies with transformed cultures, half of the cells of a single embryo were infected 4 hr after seeding with 10^6 foci-forming U of Rous-sarcoma virus/plate. For (1-^{3}H)- and (6-^{3}H) glucose experiments, 1.0 ml of medium 199 containing high specific activity (^{14}C) glucose and selectively labeled (^{3}H)glucose were added to each culture. At various intervals the medium was removed, cells killed and disrupted by sonic oscillation, and applied to filter paper for analysis by two-dimensional chromatography. After the cells were killed, the glucose concentration in samples of medium was determined by the glucostat method. In ^{3}(H)HO experiments, medium containing 200-600 mCi ^{3}(H)HO and 390 μCi(U-^{14}C)glucose/ml was added to the cells for 1 hr. The results show that both normal and transformed cells metabolize about 20% of the glucose *via* the oxidative pentose phosphate cycle. Rates for the transformed cells were twice as great as for the normal cells. However, the ratio of glucose metabolized *via* oxidative pentose cycle to the net flow of that metabolized directly to fructose-6-phosphate is about the same in normal and transformed cells. Although the rate of flow of (^{14}C)glucose into the tricarboxylic acid cycle intermediates and amino acids derived from them appears to be the same in normal and transformed cells, the rate of tritium incorporation from ^{3}(H)HO into these intermediates seems to be much higher in normal cells. It is suggested that many steps in glucose metabolism are similar in normal and virus-transformed animal cells in tissue culture.

0848 MOLECULAR WEIGHT OF RNA SUBUNITS OF ROUS SARCOMA VIRUS DETERMINED BY ELECTRON MICROSCOPY. (Eng.) Jacobson, A. B. (Max-Planck Inst. Biochem., Munich, West Germany); Bromley, P. A. *J. Vir.* 15(1):161-166; 1975.

An electron microscopic technique for the determination of RNA molecular weight is reported for the Schmidt-Ruppin strain of Rous sarcoma virus (RSV). The virus is cultivated in secondary cultures of chick embryo fibroblasts. Five days after infection, the medium is replaced at 2-hr intervals with phosphate-free Eagle's medium containing 50 μCi/ml [^{32}P] orthophosphate. Virus is collected from this replaced medium by centrifugation at 45,000 rpm for two hours at 4 C, and the RNA is extracted by standard methods and denatured with 1 mM dimethyl sulfoxide. The 33S subunit of RNA was isolated by sucrose density centrifugation and prepared for electron microscopy by a modification of the basic protein film technique. Ten microliters of RNA is added to 70 μl formamide and 10 μl triethanolamine (100 mM) and EDTA (10 mM); 10 μl cytochrome C is added after 15 min incubation at 50 C and 5 min on ice. This is pipetted onto a distilled water surface, and the cytochrome films are picked up on parlodion-coated grids. RNA of RSV was 2.86 μm long, while bacteriophage MS2 RNA was 1.12 μm after preparation in the same manner. Since the reported molecular weight of MS2 RNA is 1.2 x 10^6, the molecular weight of RNA from RSV is calculated as 3.12 x 10^6. This determination is in agreement with that of other methods, suggesting this method to be a valid technique.

0849 CELL SURFACE CHANGES AND ROUS SARCOMA VIRUS GENE EXPRESSION IN SYNCHRONIZED CELLS. (Eng.) Hale, A. H. (Dep. Microbiology, Univ. Illinois, Urbana, Ill. 61801); Winkelhake, J. L.; Weber, M. J. *J. Cell Biol.* 64(2):398-407; 1975.

Studies were conducted to determine whether changes associated with growth control and malignant transformation are linked to the cell cycle. Chicken embryo cells, infected with a temperature-sensitive

mutant of Rous sarcoma virus, were synchronized by double thymidine block. Cells were examined for cell cycle-dependent alterations in membrane function by determining the rate of transport of 2-deoxyglucose, uridine, thymidine, and mannitol. (^{3}H)2-deoxyglucose was used at 0.5 μg/ml, uridine and mannitol at 1.0 μCi/ml, and (^{3}H)thymidine at 5.0 μCi/l. Cell cycle-dependent alterations in cell surface morphology were determined by scanning electron microscopy. Cells were also examined for the ability of tumor virus gene expression to induce a transformation-specific change in membrane function; infected cells were shifted from the restrictive to the permissive temperature and monitored for a transformation-specific increase in 2-deoxyglucose transport rate. It was determined that the high rate of 2-deoxyglucose transport seen in transformed cells and the low rates of 2-deoxyglucose and uridine transport characteristic of density-inhibited cells do not occur in normally growing cells as they traverse the cell cycle. Although there are cell cycle-dependent changes in surface morphology, they are not reflected in corresponding changes in membrane function. Tumor virus gene expression can alter cell membrane function at any stage in the cell cycle and without progression through the cell cycle. It is concluded that cell surface changes associated with growth control and malignant transformation are not cell cycle dependent.

0850 ADENOVIRUS-TRANSFORMED CELLS RESTRICT HERPES SIMPLEX VIRUS REPLICATION. (Eng.) Tucker, A. G. (Dep. Microbiol., Pennsylvania State Univ., Univ. Park, Pa. 16802); Docherty, J. J. *Infect. Immun.* 11(3):556-562; 1975.

The fibroblastic-like HDC-1⁷ cell line was studied for its ability to support the growth of herpes simplex virus types 1 and/or 2 after transformation with simian adenovirus 7. Transformants of the HDC-17 cell line were produced by infecting cell suspensions with simian adenovirus 7 at a multiplicity of infection of 6 for 3 hr. HDC-17 and transformed HDC-17 cells were infected with herpes simplex virus 1 or 2 at a multiplicity of infection of 1 for 1 hr at room temperature. Samples were taken every 24 hr for 120 hr. After sonication, the samples were titrated on rabbit kidney monolayers. Cell extracts were prepared and examined for the presence of thymidine kinase and protein. Thermal stability of viral enzyme was determined by placing aliquots of cell extract at 41 C for 15 min and then assaying for residual enzyme activity. Cellular DNA was separated from viral DNA by isopycnic centrifugation. Indirect immunofluorescence was also used to determine viral presence. The results showed that the HDC-17 cell line became resistant to herpes simplex virus types 1 and 2 after transformation by simian adenovirus 7. Kinetic studies of the mechanism of resistance demonstrated that both herpes viruses were able to attach to the transformed cells and express some early genomic functions, as shown by the presence of low levels of viral thymidine kinase. Isopycnic centrifugation studies of the abortive system failed to detect viral DNA synthesis, whereas indirect immunofluorescent studies of viral proteins revealed that less than 10% of the cells contained these viral macromolecules at any given time. Collectively the data suggest that after transformation by simian adenovirus 7, these cells are altered so as to render them resistant or incapable of supporting the growth of herpes simplex virus types 1 and 2. The results further suggest that the block occurs after viral adsorption and prior to viral DNA synthesis.

0851 PRODUCTS OF COMPLEMENTATION BETWEEN TEMPERATURE-SENSITIVE MUTANTS OF SIMIAN VIRUS 40. (Eng.) Chou, J. Y. (Natl. Inst. Arthritis, Metabolism and Digestive Diseases, Bethesda, Md. 20014); Martin, R. G. *J. Virol.* 15(1):127-136; 1975.

From complementation analyses, temperature-sensitive mutants of simian virus 40 have been divided into classes A, B, C, BC, and D. Temperature-sensitive mutants of the D complementation group exhibit delayed complementation. A study was conducted of the progeny obtained after co-infection with D mutants and temperature-sensitive mutants of the other classes An hypothesis is presented to account for the delayed complementation. For thermal inactivation, virion stocks were diluted 50-fold with phosphate buffered saline and incubated at 50 C. To study the kinetics of temperature shift, confluent monolayers of CV_1 cells in Linbro plates were infected with mutants at multiplicities of infection of 2 to 5. After a 2-hr adsorption at 33 C, fresh medium was added. At appropriate times, cultures were shifted to 40 C. At 96 hr postinfection, all cultures were frozen, thawed, and titered. To study the products of complementation, cultures of CV_1 cells in Linbro plates were co-infected with wild-type and mutant virions. The progeny were plated at the permissive temperature and plaques were picked at random, suspended in 2.0 ml of phosphate buffered saline, and tested for their ability to grow at the restrictive temperature. Analysis of the results led to the hypothesis that delayed complementation is not true complementation, but the result of a very low level of leakiness, followed by phenotypic mixing of the progeny D mutants. This is consistent with the proposal that D mutants are defective in uncoating. It was also observed that fresh medium suppresses the growth of D mutants at the restrictive temperature.

0852 UPTAKE OF SV40 ANTIGEN INTO CHICK ERYTHROCYTE NUCLEI IN HETEROKARYONS. (Eng.) Rosenqvist, M. (Medical Nobel Inst., S-104 01 Stockholm 60, Sweden); Stenman, S.; Ringertz, N. R. *Exp. Cell Res.* 92(2):515-518; 1975.

The intracellular migration of the nuclear simian virus 40 (SV40) T antigen in heterokaryons between SV40-transformed cells and chick RBC was investigated. WiSV40, a human SV40-transformed cell line, and HeLa cells were cultivated in Eagle's minimum essential medium supplemented with 10% calf serum and antibiotics. Monolayer cells were fused with suspended RBC using UV-inactivated Sendai virus. In some experiments the fusion was done in suspension by mixing

10^6 WiSV40 cells with 100 x 10^6 RBC and 8,000 hemagglutinating units of virus in Earle's BSS. Cells were stained for SV40 T antigen by indirect immunofluorescence using antisera from hamsters bearing SV40-induced tumors. In heterokaryons examined early after fusion, the RBC nuclei did not react with the T antiserum and appeared as nonfluorescent spaces against the cytoplasmic background. At later times, the nuclei gradually became positive for T antigen. When the preparations were scored only for strongly positive RBC nuclei, 4% of the chick nuclei were positive for T antigen six hours after fusion; 17% were positive 12 hr after fusion, and 65% were positive at 24 hr. In several experiments, almost all RBC were positive 45 hr after fusion. When RBC and SV40-transformed cells were fused in suspension and harvested 21, 45, and 69 hr after fusion, quantitative immunofluorimetric measurements showed that the T antigenicity of the nuclei in heterokaryons increased during the first two days, whereas that of the WiSV40 nuclei remained constant. These results show that the SV40 T antigen migrated into the chick RBC nucleus as it reactivated in heterokaryons of SV40-transformed cells and RBC. The overall kinetics of T antigen accumulation in the RBC nucleus was similar to that found with other nuclear proteins and antigens in other types of chick RBC heterokaryons. The migrations of T antigen into reactivating nuclei (which cannot be transformed or infected with SV40) leads to the conclusion that nuclear uptake of SV40-specific T antigen is independent of the permissiveness and transformability of the mammalian cell.

0853 STRUCTURAL PROTEINS OF SIMIAN VIRUS 40. I. HISTONE CHARACTERISTICS OF LOW-MOLECULAR-WEIGHT POLYPEPTIDES. (Eng.) Pett, D. M. (Dep. Bacteriol. Immunol., Univ. North Carolina, Chapel Hill); Estes, M. K.; Pagano, J. S. *J. Virol.* 15(2): 379-385; 1975.

The DNA-associated polypeptides of simian virus 40 (SV40), VP4 (molecular wt 14,000), VP5 (molecular wt 12,000) and VP6 (molecular wt 11,000) were investigated to determine if they are virus-specified gene products or cellular histones which become associated with the virus DNA and encapsidated. After extraction from purified SV40 with dilute acids, these three polypeptides co-electrophoresed on low pH polyacrylamide gels with monkey kidney cell histones F3, F2b, and $F2a_1$. No virus polypeptide co-electrophoresed with histone F1. Polypeptides VP4, 5, and 6 lacked tryptophan, and only VP4 contained cysteine, as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis of virus labeled *in vivo* with ^{3}H-lysine and either ^{14}C-tryptophan or ^{35}S-cystine. All of the capsid polypeptides VP1, 2, and 3 contained tryptophan whereas only VP1 and 2 contained cysteine. In addition, VP4, 5, and 6 were rich in arginine and lysine when compared with virus labeled with a mixture of amino acids. Analysis of virus grown in cells labeled prior to infection showed that VP4, 5, and 6 were labeled fivefold greater than the major capsid polypeptide, VP1, which indicates that they were partially derived from preexisting cell histones. Based on these data and on previously determined molecular weight estimates, it is concluded that VP4, 5, and 6 are histones F3, F2b and $F2a_1$, respectively, although the possibility that SV40 contains a small amount of $F2a_2$ cannot be excluded.

0854 POSTTRANSCRIPTIONAL SELECTION OF SIMIAN VIRUS 40-SPECIFIC RNA. (Eng.) Khoury, G. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.) Howley, P.; Nathans, D.; Martin, M. *J. Virol.* 15(2) 433-437; 1975.

The viral-specific RNA present in the nucleus and the cytoplasm of simian virus 40 (SV40)-infected monkey kidney cells was examined. Increasing amounts of cytoplasmic RNA from BSC-1 cells obtained 48 hr after infection with SV40 were incubated with small amounts of ^{32}P-labeled fragments of either the plus or minus SV40 DNA strand. After 36 hr, the reaction mixtures were analyzed by hydroxyapatite chromatography to determine the percent DNA in hybrid molecules. There was extensive transcription of both DNA strands. These symmetrically transcribed sequences were localized in the nucleus of infected cells, whereas only the true early and late SV40 transcripts were found in the cytoplasm. These results suggest that selective posttranscriptional degradation and/or transport occurred after transcription of the viral DNA. On the basis of hybridization experiments with cytoplasmic RNA and the separated strands of the SV40 *Haemophilus influenza* (*Hin*)-fragments, the early SV40 region appeared to include all of *Hin* fragments A, H, I, and B (48% of the genome), whereas the late region was represented in *Hin* fragments C, D, E, K, F, J, and G (52% of the genome). This study provides a more precise location of the early and late regions of the SV40 genome. The proximal junction of these segments lies very close to the *Hin* AC junction and, therefore, very close to the initiation site for DNA replication. Whereas the relationship between these sites is presently unknown, it seems likely that their proximity may have some functional significance.

0855 CHROMATIN STRUCTURE: DEDUCED FROM A MINICHROMOSOME. (Eng.) Griffith, J. D. (Stanford Univ. Sch. Med., Calif.). *Science* 187(4182):1202-1203; 1975.

A very small chromosome isolated from monkey cell cultures lytically infected with simian virus (SV40) was studied to determine the U fiber packing ratio (calculated by dividing the countour lengths of deproteinized DNA by that of the native minichromosome). The results were compared with a previously published model of chromatin structure which proposed that the U fiber is a flexibly joined chain of repeating U 100 Å in length and estimated that the length of duplex DNA contained in a given length of U fiber would be 68:1. After permissive monkey cell cultures were infected with SV40, a pool of circular viral DNA molecules accumulated in the nucleus of the infected cell. The SV40 DNA was isolated as a nucleoprotein complex. More than 95% of the proteins in the complex were identified as cellular histones which were present in a 1:1 ratio

with the viral DNA. The structure, nuclear location, and stoichiometry of histones in this SV40 complex suggest that it is a very small chromosome. The small SV40 chromosomes were prepared by modifications of a procedure for isolating interphase chromatin including the maintenance of physiologic salt concentrations to preserve the native structure. Purified small chromosomes were fixed and visualized by high resolution electron microscopy. Loops of a fiber 110 Å in diameter and 2100 Å in length were observed. The loops were either open circles or circles twisted about themselves. The defined dimensions of the small SV40 chromosome and the DNA contained in it allowed a direct measurement of the packing ratio in the fiber, giving a value of 7:1. A 10-fold decrease in ionic strength caused the compact U fiber to relax into a flexible string of 21 beads, each 110 Å in diameter and joined by bridges roughly 20 Å in diameter and 130 Å long. It is suggested that, when the DNA of the small chromosomes is complexed with histone proteins, the DNA is condensed seven-fold to form a 2100 Å length of the typical U fiber and that this condensation occurs in blocks, each block or bead containing 170 base pairs of DNA and each bridge about 40. It is concluded that DNA in the U fiber exists in two states, bead DNA and bridge DNA, and that the values determined in this study are similar to those of eukaryotic chromosomes and consistent with the previously published model of chromatin structure.

0856 RESPONSE OF CELL SURFACE GLYCOSYL TRANSFERASES TO DIBUTYRYL ADENOSINE-3',5' CYCLIC MONOPHOSPHATE IN VIRUS-TRANSFORMED AND NORMAL CELLS. (Eng.) Sudo, T. (Inst. Med. Sci., Univ. Tokyo, Japan); Onodera, K. *Exp. Cell Res.* 91(1):191-199; 1975.

The response of galactosyl and sialyl transferases in the plasma membrane of SV40-transformed and normal C3H-2K mouse cells to dibutyryl adenosine-3',5' cyclic monophosphate (db-cAMP) was investigated. The complete enzyme system contained the following in a volume of 0.15 ml: 10 µl 0.1M $MnCl_2$, 10 µl 0.1 M $MgCl_2$, 20 µl phosphate buffer saline (pH 7.2) or exogenous acceptors (450 µg protein), 10 µl of radioactive nucleotide sugar, 0.1 ml of cell suspension in Dulbecco's medium (300 µg protein) and 10 µl db-cAMP. The glycosyl transferases of the virus-transformed cells showed a marked response to db-cAMP. The transfer of sialic acid to the endogenous acceptor was slightly stimulated at low concentration of db-cAMP. Sialyl and galactosyl glycoprotein transferases of the virus-transformed cells were both inhibited by 0.5 mM db-cAMP. Surface glycosyl transferases of normal cells, however, were not inhibited. Ninety-five percent of the cells in Dulbecco's medium supplemented with Mg^{++} were viable during the reaction at least for one hr. A differential response of galactosyl transferase was observed in the plasma membrane preparations of normal and virus-transformed cells. Galactosyl transferase was stimulated at 0.1 mM db-cAMP in the viral transformed cells while in the normal cells it was slightly inhibited. By contrast, microsomal galactosyl transferases from both cell types were stimulated by db-cAMP to a slightly different degree. cAMP was not effective on microsomal sialyl transferases. The results demonstrate that the surface membranes of normal and virus-transformed cells show a remarkable differential response to db-cAMP.

0857 CELL PROPERTIES AFTER REPEATED TRANSPLANTATION OF SPONTANEOUSLY AND OF SV40 VIRUS TRANSFORMED MOUSE CELL LINES. I. GROWTH IN CULTURE. (Eng.) McFarland, V. W. (Natl. Cancer Inst., Bethesda, Md.); Mora, P. T.; Schultz, A.; Pancake, S. *J. Cell. Physiol.* 85(1):101-112; 1975.

Spontaneously transformed or simian 40 (SV40)-transformed AL/N mouse cell lines were passed repeatedly through syngeneic mice. Cell lines were reestablished in culture from minced pieces of tumors in the presence of concentrated fetal calf serum or from tumor cells dispered by trypsin. Measurements of growth in tissue culture on substratum showed no signficant difference between any of the transformed cell lines. SV40-transformed cells and derivative cells had a low anchorage requirement for growth. The greatest anchorage requirement for growth was in normal untransformed cells and in derivatives from the spontaneously transformed cells which were established from minced tumors. The spontaneously transformed cells and all derivative cells had high tumorigenicity *in vivo* ($TD_{50} < 10^2$). The SV40-transformed cells had no observable tumorigenicity ($TD_{50} > 10^8$) except when injected into irradiated mice ($TD_{50} = 10^7$). The derivative cell lines obtained from such a tumor had increased tumorigenicity (TD_{50} = 1-5 x 10^5 in the immunocompetent mice, 5 x 10^4 in the irradiated mice). The SV40-transformed derivative cells maintained their SV40 specific T antigen and their susceptibility to lysis by specific antiserum.

0858 A COMPARISON OF GLYCOSYLTRANSFERASE ACTIVITIES AND MALIGNANT PROPERTIES IN NORMAL AND TRANSFORMED CELLS DERIVED FROM BALB/C MICE. (Eng.) Patt, L. M. (Coll. Med., Univ. Arizona, Tucson); Van Nest, G. A.; Grimes, W. J. *Cancer Res.* 35(2):438-441; 1975.

In order to correlate the biochemical aspects of the cell surface with changes leading to cancer, several normal, spontaneously transformed, and virally transformed cell lines derived from inbred BALB/c mice were studied. The ability of cell suspensions to catalyze the incorporation of nucleotide sugars into complex polysaccharides was compared. These cells were previously characterized for concanavalin A-induced agglutinability, tumorigenicity, and malignancy. All of the cell lines tested catalyzed transfer of cytosine 5'-monophosphate *N*-acetylneuraminic acid, uridine 5'-diphosphate galactose, uridine 5'-diphosphate *N*-acetylgalactosamine, uridine 5'-diphosphate *N*-acetylglucosamine, uridine 5'-diphosphate glucose, and guanidine 5'-diphosphate mannose into glycoproteins and glycolipids. The highest activities were found in one line of normal cells. It is suggested that the process of establishment leads to a lowering of glycosyltransferase levels detected in this system. While some transformed cell lines showed alterations in transferase

levels, others could not be distinguished from normal cells. Normal cells, transformed cells that cause tumors that regress, and transformed cells that cause tumors that kill an immunologically competent host showed growth-dependent changes in transferase activities. No evidence was obtained to support the hypothesis that cell surface glycosyltransferases play a direct role in contact inhibition or cell-to-cell interactions. It is concluded that determining the ability to catalyze carbohydrate transfer is insufficient for predicting the tumorigenic and malignant properties of a cell line.

0859 CELL SURFACE CHANGES CORRELATED WITH DENSITY-DEPENDENT GROWTH INHIBITION. GLYCOSAMINOGLYCAN METABOLISM IN 3T3, SV3T3, AND CON A SELECTED REVERTANT CELLS. (Eng.) Roblin, R. (Harvard Med. Sch., Boston, Mass.); Albert, S. O.; Gelb, N. A.; Black, P. H. *Biochemistry* 14(2):347-357; 1975.

A $^{35}SO_4$-labeling/chromatography technique has been developed which facilitates quantitation of sulfated glycosaminoglycan (GAG) synthesis in mammalian cell cultures. The technique has been used to compare sulfated GAG biosynthesis, degradation, and turnover in three related cell lines with differing degrees of density-dependent growth inhibition *in vitro:* BALB/c 3T3, simian virus 40-transformed 3T3 (SV3T3), and SV3T3 revertant cells. SV3T3 cells incorporated 2 to 5-fold less $^{35}SO_4$ than their untransformed counterparts. SV3T3 revertant cells, which show partial reversion to low saturation density *in vitro*, showed a 2.5- to 8-fold increase in cell-associated sulfated GAG compared with the parental SV3T3 cells from which they were selected. Mild trypsin treatment of $^{35}SO_4$-labeled cells removed 68-84% of the cellular sulfated GAG, suggesting that at least this proportion of the total cellular sulfated GAG was located at the cell periphery. Removal of $^{35}SO_4$-labeled cells from the Petri dish with a Ca^{++} selective chelating agent revealed a fraction of GAG that remained tightly bound to the dish. A higher proportion of the total cell-associated sulfated GAG remained attached to the Petri dish in untransformed and revertant cell cultures compared with that present in transformed cell cultures. It is suggested that a correlation exists between a relatively high level of GAG synthesis and low saturation density in cell cultures.

0860 METABOLIC PROPERTIES OF SUBSTRATE-ATTACHED GLYCOPROTEINS FROM NORMAL AND VIRUS-TRANSFORMED CELLS. (Eng.) Culp, L. A. (Case West. Reserve Univ. Sch. Med., Cleveland, Ohio); Terry, A. H.; Buniel, J. F. *Biochemistry* 14(2):406-412; 1975.

BALB/c 3T3, SV40-transformed 3T3 cells, and concanavalin A revertant variants of transformed cells leave a layer of glycoprotein on the culture substrate upon ethylenebis(oxyethylenenitrilo)tetraacetic acid-mediated removal of cells. The metabolic properties of this glycoprotein have been studied, including its accumulation on the substrate, rate of synthesis, and turnover as a function of growth of contact-inhibited and virus-transformed cells. Pulse and cumulative radiolabeling experiments with glucosamine and leucine precursors established that this glycoprotein accumulates on the substrate in growing cultures until cells have completely covered the substrate. The synthesis and/or deposition of the material diminished dramatically in cultures whose substrates had been completely covered with cells, even though the contact-inhibited cell lines continued to make cell-associated and medium-secreted glycoproteins, and transformed cells continued to divide and form multilayered cultures. Pulse-chase analysis using long periods of pulsing with radioactive leucine showed that these glycoproteins are deposited directly on the substrate by cells and not subsequent to secretion into the medium. The glycoprotein that accumulated during long pulses was stably adherent to the substrate and displayed little appreciable turnover during three days of chasing of either sparse or dense cultures. Short-term pulse-chase analysis with leucine revealed two metabolically different pools of material, one which turns over very rapidly with a half-life of two to three hr and a second pool which is stably deposited on the substrate and whose proportion increased with the length of the radiolabeling period. It is concluded that there are no appreciable differences in the metabolic properties of the glycoprotein observed in the three cell types studied during growth on a plastic substrate. These results are discussed with regard to the implicated roles of these glycoproteins in mediating adhesion of normal and virus-transformed cells to the substrate.

0861 HUMAN ADENOVIRUS-INDUCED CEREBELLAR NEOPLASMS [abstract]. (Eng.) Mukai, N. (Retina Found., Boston, Mass.); Murao, T. *J. Neuropathol. Exp. Neurol.* 34(1):93; 1975.

0862 INCIDENCE AND MORPHOLOGY OF INTRACRANIAL TUMORS INDUCED BY THE BRATISLAVA-77 STRAIN OF AVIAN SARCOMA VIRUS IN PERINATAL AND ADULT RATS [abstract]. (Eng.) Copeland, D. D. (Natl. Inst. Environ. Health Sci., Research Triangle Park, N.C.); Vogel, F. S.; Self, D. J.; Bigner, D. D. *J. Neuropathol. Exp. Neurol.* 34(1):100; 1975.

0863 HOST INDUCED ALTERATION OF AVIAN SARCOMA VIRUS B-77 GENOME. (Eng.) Shoyab, M. (Univ. California Los Angeles Sch. Med.); Markham, P. D.; Baluda, M. A. *Proc. Natl. Acad. Sci. USA* 72(3):1031-1035; 1975.

0864 EXPRESSION OF TUMOR-SPECIFIC SURFACE ANTIGENS ON CELLS INFECTED WITH TEMPERATURE-SENSITIVE MUTANTS OF AVIAN SARCOMA VIRUS. (Eng.) Kurth, R. (Imp. Cancer Res. Fund Lab., London, England); Friis, R. R.; Wyke, J. A.; Bauer, H. *Virology* 64(2):400-408; 1975.

0865 COMPARATIVE CHEMICAL PROPERTIES OF AVIAN ONCORNAVIRUS POLYPEPTIDES. (Eng.) Herman, A. C. (Duke Univ. Med. Cent., Durham, N.C.); Green, R. W.; Bolognesi, D. P.; Vanaman, T. C. *Virology* 64(2):339-348; 1975.

0866 EXPRESSION OF THE MAJOR VIRAL GLYCOPROTEIN OF AVIAN TUMOR VIRUS IN CELLS OF CHF(+) CHICKEN EMBRYOS. (Eng.) Halpern, M. S. (Wistar Inst. Anat. Biol., Philadelphia, Pa.); Bolognesi, D. P.; Friis, R. R.; Mason, W. S. *J. Virol.* 15(5): 1131-1140; 1975.

0867 PARTIAL PURIFICATION AND PROPERTIES OF DNA POLYMERASES FROM JLS-V10 CELLS. (Eng.) Bandyopadhyay, A. K. (Natl. Cancer Inst., Frederick, Md.). *Arch. Biochem. Biophys.* 166(1):83-93; 1975.

0868 AFFINITY CHROMATOGRAPHY OF VIRAL DNA POLYMERASES ON PYRAN-SEPHAROSE. (Eng.) Chirikjian, J. G. (Sch. Med., Georgetown Univ., Washington, D.C.); Rye, L.; Papas, T. S. *Proc. Natl. Acad. Sci. USA* 72(3):1142-1146; 1975.

0869 EPSTEIN-BARR VIRUS: COMPARISON OF DIFFERENT STRAINS FOR THEIR BIOLOGICAL ACTIVITIES *IN VITRO*. (Eng.) Furukawa, T. (Inst. Cancer Res., Philadelphia, Pa.). *Res. Commun. Chem. Pathol. Pharmacol.* 10(3):543-553; 1975.

0870 ARGININE REQUIREMENT FOR FROG VIRUS 3 DEVELOPMENT. (Eng.) Aubertin, A.-M. (Groupe de Recherches de l'I.N.S.E.R.M. Path. Inf. Vir. Lab. Virol. Univ. Louis Pasteur, 3 rue Koeberle, 67000 Strasbourg, France). *Virology* 63(2):573-576; 1975.

0871 MASTOCYTOMA CELL MIGRATION *IN VITRO*: INHIBITION BY MIF-CONTAINING SUPERNATANTS. (Eng.) Cohen, M. C. (Univ. Connecticut Health Cent., Farmington); Zeschke, R.; Bigazzi, P. E.; Yoshida, T.; Cohen, S. *J. Immunol.* 114(5):1641-1643; 1975.

0872 VIREMIC RESPONSES OF GENETICALLY SUSCEPTIBLE AND RESISTANT CHICKENS TO EXPERIMENTAL INFECTION WITH ACUTE, MILD, OR BOTH STRAINS OF MAREK'S DISEASE HERPESVIRUS. (Eng.) Cho, B. R. (Dep. Vet. Sci., Washington Agric. Exp. Stn., Pullman, Wash.). *Avian Dis.* 19(1):67-74; 1975.

0873 PATHOGENESIS OF NEURAL LESIONS IN MAREK'S DISEASE. II. TRANSMISSION OF NEURAL LESIONS BY SPLEEN CELLS FROM ILL DONORS. (Ger.) Hoffmann-Fezer, G. (Institut fur Biologie, Abt. Nuklearbiologie D-8042 Neuherberg/Munchen, Ingolstadter Landstrasse 1, West Germany); Schmahl*, W.; Hoffmann, R. *Z. Immunitaetsforsch.* 150(4):300-308; 1975.

0874 CHARACTERISTICS OF MAMMARY TUMOR CULTURES FROM FOUR MOUSE STRAINS INFECTED WITH MAMMARY TUMOR VIRUS. (Eng.) Yagi, M. J. (Dep. Bacteriol. Immunol., Univ. California, Berkeley). *Cancer Res.* 35(2):370-373; 1975.

0875 PROPERTIES OF MOUSE LEUKEMIA VIRUSES. XII. PRODUCTION OF SUBSTANTIAL AMOUNTS OF FRIEND LEUKEMIA VIRUS BY A SUSPENSION TISSUE CULTURE LINE (EVELINE SUSPENSION CELLS). (Ger.) Seifert, E. (Max-Planck-Institut fuer Virusforschung, Tubingen, West Germany); Claviez, M.; Frank, H.; Hunsmann, G.; Schwarz, H.; Schafer, W. *Z. Naturforsch. [C]* 30(9/10):698-700; 1975.

0876 LACK OF REQUIREMENT OF REVERSE TRANSCRIPTASE FUNCTION FOR THE ACTIVATION OF MURINE LEUKEMIA VIRUS BY HALOGENATED PYRIMIDINES. (Eng.) Pitha, P. M. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.); Pitha, J.; Rowe, W. P. *Virology* 63(2):568-572; 1975.

0877 SECONDARY SCANNING ELECTRON MICROSCOPY OF CELLS INFECTED WITH MURINE ONCORNAVIRUSES. (Eng.) Panem, S. (Div. Biol. Sci., Univ. Chicago, Ill.); Kirsten, W. H. *Virology* 63(2): 447-458; 1975.

0878 CHARACTERIZATION OF DNA POLYMERASE AND RNA ASSOCIATED WITH A-TYPE PARTICLES FROM MURINE MYELOMA CELLS. (Eng.) Robertson, D. L. (Div. Biol. Biomed. Sci., Washington Univ., St. Louis, Mo.); Baenziger, N. L.; Dobbertin, D. C.; Thach, R. E. *J. Virol.* 15(2):407-415; 1975.

0879 MYXOVIRUS-LIKE PARTICLES IN UNTREATED UNDIFFERENTIATED BURKITT'S TYPE LYMPHOMA [abstract]. (Eng.) Popoff, N. (Univ. Miami Sch. Med., Fla.); Malinin, T.; Cullen, R. *J. Neuropathol. Exp. Neurol.* 34(1):111; 1975.

0880 FINE STRUCTURE OF FIBROBLASTS TRANSFORMED *IN VITRO* BY POLYOMA VIRUS [abstract]. (Eng.) Singh, B. B. (Med. Coll. Georgia, Augusta); Schuster, G. S.; McKinney, R. V.; Volkman, K. *Lab. Invest.* 32(3):456-457; 1975.

0881 AMINO-TERMINAL SEQUENCE OF BABOON TYPE C VIRUS p30. (Eng.) Oroszlan, S. (Flow Lab., Inc., Rockville, Md.); Summers, M.; Gilden, R. V. *Virology* 64(2):581-583; 1975.

0882 INDUCTION OF AN INAPPARENT "FOAMY" VIRUS IN HAMSTER CELLS TRANSFORMED BY SV40. (Fre.) Rossignol, J.-M. (Institut de Recherches Scientifiques sur le Cancer, B. P. no. 8, 94800 Villejuif, France); Kress, M.; de Vaux Saint Cyr, C. *C. R. Acad. Sci. [D] (Paris)* 281(15):1145-1148; 1975.

0883 EFFECT OF HOST AGE AND VIRUS DOSE ON TUMOR INCIDENCE, LATENCY AND MORPHOLOGY IN SYRIAN GOLDEN HAMSTERS INOCULATED INTRAVENOUSLY WITH SV 40 [abstract]. (Eng.) Diamandopoulos, G. T. (Harvard Med. Sch., Boston, Mass.); McLane, M.-F. *Am. J. Pathol.* 78(1):60a; 1975.

0884 QUANTITATION OF RNA TUMOR VIRUSES BY LASER BEAT FREQUENCY LIGHT SCATTERING SPECTROSCOPY (LBFS): REVERSE TRANSCRIPTASE ACTIVITY PER VIRION FOR AVIAN MYELOBLASTOSIS (AMV-BAI STRAIN) AND MURINE LEUKEMIA (MuLV-RAUSCHER) VIRUSES [abstract]. Salmeen, I. (Sci. Res. Staff, Ford Mot. Co., Dearborn, Mich.); Rimai, L.; Liebes, L.; Rich, M. A.; McCormick, J. J. *Biophys. J.* 15(2):202a; 1975.

0885 QUANTITATION OF RNA TUMOR VIRUSES BY SPECTROSCOPY OF DENSITY GRADIENT-GELS [abstract]. (Eng.) Liebes, L. (Michigan Cancer Found., Detroit); Retzel, E.; Rich, M. A.; McCormick, J. J.; Salmeen, I.; Rimai, L. *Biophys. J.* 15(2):201a; 1975.

0886 INCREASED MUTAGENICITY OF VIRUS IN THE PRESENCE OF CADMIUM SALT. (Rus.) Zasukhina, G. D. (Inst. General Genetics of the Acad. Sciences of the USSR, Moscow, USSR); Shalupova, N. V.; Shvetsova, T. P.; Lomanova, G. A. *Dokl. Akad. Nauk SSSR* 224(5):1189-1191; 1975.

0887 SAMPLING STATISTICS IN SEARCHING FOR VIRUS PARTICLES IN HUMAN TUMORS. IMPROVEMENT USING AN AUTOMATED HIGH VOLTAGE ELECTRON MICROSCOPE WITH PATTERN RECOGNITION IMAGE PROCESSING [abstract]. (Eng.) Parsons, D. F. (Roswell Park Mem. Inst., Buffalo, N.Y.); Whaley, D. A. *Biophys. J.* 15(2): 202a; 1975.

0888 COMPARISON OF TRYPTIC PEPTIDE MAPS OF SOME ONCOGENIC VIRUS PROTEINS [abstract]. (Eng.) Benson, J. R. (Stanford Univ., Calif.). *Diss. Abstr. Int. B* 35(9):4357; 1975.

See also:

* (Rev): 606, 607, 608, 623, 635, 636, 637
* (Chem): 674, 701
* (Immun): 897, 898, 905, 906, 907, 911, 917, 918, 919, 925, 926, 927, 928, 937, 938, 939, 940, 941, 950, 957, 958, 968, 970, 981

0889 IMMUNOLOGICAL STUDIES WITH MURINE MASTOCYTOMAS. (Eng.) Minard, P. (Johns Hopkins Univ., Baltimore, Md.). *Diss. Abstr. Int. B.* 35(9): 4515-4516; 1975.

Mouse mastocytoma cells were studied with the aim of establishing a model system for investigation of aspects of mast cell involvement in the immediate hypersensitivity reaction. An antibody depletion technique was developed to study binding of IgE antibody to Furth mastocytoma cells. IgE antibody binding was dependent on salt concentration, time and temperature. HC cells (a subline of the Furth mastocytoma) had 100 IgE antibody receptors/cell. The average association constant was 10^{11} M^{-1}, and the forward rate constant was an estimated 7.7×10^{7} min^{-1} sec^{-1}. HC mastocytoma cells depleted IgE antibody from rat, dog and human sera; this indicates that binding of IgE to mast cells may be less specific, and that binding and histamine release are distinct events.

0890 INITIATION-PROMOTION SKIN CARCINOGENESIS AND IMMUNOLOGICAL COMPETENCE. (Eng.) Curtis, G. L. (Univ. Nebraska Medical Center, 42nd and Dewey, Omaha, Nebr. 68105); Stenback, F.; Ryan, W. L. *Proc. Soc. Exp. Biol. Med.* 150(1):61-64; 1975.

Host immune response during different phases of initiation-promotion carcinogenesis was studied using strain A mice as the host, 7,12-dimethylbenzanthracene (DMBA) as the initiator, and croton oil as the promotor. Tests for immune response were based on time of retention of C3H skin allografts or on response of cultured host spleen lymphocytes to phytohemagglutinin (PHA) or pokeweed mitogen (PWM) stimulation as measured by ^{3}H-thymidine uptake. For the skin grafting tests, DMBA was applied in the amount of 100 µg in acetone to the interscapular area of the skin, while the croton oil was applied 10 days later in the amount of 20 µl in acetone twice a week for 30 weeks. Of the mice so treated, one group (57%) developed tumors; the remaining group (33%) did not. Other groups of mice were treated with each agent alone or with neither agent. Allografts on the groups of mice treated with both DMBA and croton oil and bearing tumors, or treated with DMBA alone, were retained longer than were grafts on groups treated with both DMBA and croton oil and not bearing tumors, or untreated. These findings indicate that DMBA is immunosuppressive and that animals bearing tumors have a reduced cell-mediated immunity. For the lymphocyte stimulation tests, groups of mice were treated with DMBA alone, croton oil alone, chlorophenesin alone, chlorophenesin plus croton oil, or were untreated. The chlorophenesin was given i.p. in the amount of 5 mg. DMBA inhibited both PHA and PWM stimulation of thymidine incorporation, while croton oil enhanced PWM stimulation without significantly affecting PHA stimulation. This was interpreted as a reflection of stimulation of only B cells by croton oil, since PHA is known to stimulate T cells while PWM stimulates both T and B cells. The chlorophenesin inhibited the stimulation of mitogenesis by PWM. However, in additional experiments in which mice receiving both DMBA and croton oil were also treated with 5 mg. chlorophenesin i.p. twice a week at the same time that the croton oil was applied, tumor formation was reduced by 47%. It was suggested that chlorophenesin may inhibit initiation-promotion carcinogenesis by raising intracellular levels of cyclic AMP and altering the immune competence of the host.

0891 THE ENDOCRINE ROLE OF THE THYMUS AND ITS HORMONE, THYMOSIN, IN THE REGULATION OF THE GROWTH AND MATURATION OF HOST IMMUNOLOGICAL COMPETENCE. (Eng.) White, A. (Syntex Res., Palo Alto, Calif.); Goldstein, A. L. *Adv. Metab. Disord.* 8:359-374; 1975.

Thymosin, a protein hormone of the thymus gland, was purified from bovine thymus gland to yield an acidic protein, free of carbohydrate, lipid, and nucleotides, and having a molecular weight of 12,000 ± 200. A thymosin fraction with similar chemical and biological properties was also prepared from human thymus glands. The essential roles of the hormone are reflected in two established roles of the thymus gland, namely, provision in early postnatal life of lymphoid cells that are exported from the thymus to peripheral lymphoid structures and which become a cell population that endows the neonatal organism with immunological competence, and secretion from thymic epithelial cells of one or more hormones, one of which is the thymosin. These humoral factors apparently function by acting on both stem cells (predetermined T cells) and thymus-derived cells to activate or derepress a cell that has all the potential to function as a mature immunologically competent lymphocyte. In addition, thymosin may act to reverse aberrations of lymphoid cell functions, such as proliferation of aberrant thymocytes. The different roles of thymosin suggested possible practical applications to diseases reflecting failure of development or maintenance of adequate immunological competence. In a 5½-year-old girl with thymic hypoplasia, impaired immune synthesis and low lymphocyte count, treatment with thymosin brought about increased peripheral T-cell rosette formation, increased lymphocyte level, and acquisition of delayed hypersensitivity skin reactivity, but unimproved *in vitro* lymphocyte response in phytohemagglutinin-stimulation and mixed lymphocyte interaction tests. Use of thymosin in leukemic and Hodgkin's disease patients brought about remarkable increases in lymphocyte count, rises in immunoglobulin levels, return of delayed hypersensitivity, and improvement in general condition. The result of these initial clinical studies encourage further exploration of practical utility of thymosin and perhaps other thymic fractions in selected pathophysiological states.

0892 NEOPLASIA IN KIDNEY TRANSPLANT RECIPIENTS. (Eng.) Sterioff, S. (Baltimore City Hosp. 4940 Eastern Ave., Baltimore, Md. 21224); Rios, C. N.; Zachary, J. B.; Williams, G. M. *Am. J. Surg.* 130(5):622-626; 1975.

Neoplasms appearing in therapeutically immunosuppressed kidney transplant recipients are described, and the observations are considered with respect to

hypothetical explanations of impaired immunosurveillance, prolonged antigenic stimulation of the lymphoreticular system, and increased susceptibility to oncogenic viruses. Malignant tumors occurred in 6/216 patients. The mean time of appearance of tumors after transplantation was 25 months (range, 14 to 38 months). The immunosuppressants used were azothioprine, azothioprine plus prednisone, or azothioprine plus prednisone and cyclophosphamide. Five patients had epithelial cancers (two carcinomas, metastatic to the brain; "immunoblastoma", involving the lung; breast carcinoma; multiple squamous carcinoma of the skin), and one had a mesenchymal tumor (carcinoid of the stomach). The tumors occurring in the carcinoid patient and one of the brain carcinoma patients were considered incidental. Evaluation of the lymph nodes, spleen, bone marrow, and gastrointestinal lymphatic aggregates in those patients which were subjected to autopsy showed a generalized lymphoid depletion, which was related to the prolonged immunosuppressive therapy. It was recommended that treatment of renal transplant patients bearing tumors consist of standard surgical approach, discontinuation of immunosuppressive drugs, and removal of allograft. Continuation of immunosuppression for maintenance of renal function was considered to increase the likelihood of recurrence of the tumor and to be used only if the patient is aware of the risk and views return to chronic dialysis, an alternative worse than further occurrence of tumor. It was concluded that a single explanation for the increased incidence of neoplasms in immunosuppressed allograft recipients is not possible and that the complex multifactorial biologic aberration can only be partially understood at present.

0893 IMMUNOLOGICAL ESCAPE MECHANISM IN SPONTANEOUSLY METASTASIZING MAMMARY TUMORS. (Eng.) Kim, U. (Roswell Park Mem. Inst., Buffalo, N.Y.): Baumler, A.; Carruthers, C.; Bielat, K. *Proc. Natl. Acad. Sci. USA* 72(3):1012-1016; 1975.

The relationship between tumor cell immunogenicity and metastasizing capacity was investigated in two spontaneously metastasizing non- or weakly-immunogenic mammary carcinomas (SMT-2A and TMT-50) and a spontaneously metastasizing mammary carcinoma induced with 7,12-dimethylbenz(a)-anthracene in a bacille Calmette-Guérin (BCG)-inoculated rat. They were matched according to the degree of structural differentiation and growth rate with three nonmetastasizing, immunogenic mammary carcinomas (MT-W9B, MT-W9A and MT-91) also induced with methyl-cholanthrene and maintained in the same strain of rats. Tumor transplantation was performed by inoculation of 0.1 ml of fine tumor mince into the right mammary gland. All spontaneously metastasizing tumors had little or no demonstrable glycocalyx, while all nonmetastasizing tumors had a thick glycocalyx. There was a direct relationship between the glycocalyx and immunogenicity, and an inverse relationship with the metastasizing capacity of tumor cells, properties which can be quantitated by levels of the plasma membrane marker enzyme 5'-nucleotidase (EC 3.1.3.5;5'-ribonucleotide phosphohydrolase) activity. Both metastasizing and nonmetastasizing mammary tumors had a common soluble cell surface antigen. In addition, there was another membrane-bound antigen in the nonmetastasizing, immunogenic tumor cell surface which presumably was the tumor specific transplantation antigen. This antigen was immunobiologically unique, but seemed immunochemically related to the common soluble antigen. It is postulated that the lack of an immunogenic coat and/or the presence of solubilized tumor cell surface antigen in the blood may provide an immune escape mechanism for tumor cells by interfering with cell-mediated immune response of tumor hosts, leading to their dissemination.

0894 AN EXPERIMENTAL MODEL FOR EVALUATION OF FACTORS IN TUMOR ESCAPE FROM IMMUNOLOGICAL ATTACK. (Eng.) Yutoku, M. (Dep. Immunol. Res., Roswell Park Mem. Inst., Buffalo, N.Y.); Fuji, H.; Grossberg, A. L.; Pressman, D. *Cancer Res.* 35(3): 734-739; 1975.

DBA/2 mice were inoculated s.c., i.p. or i.v. with BALB/c mouse myeloma cells (MOPC-21) to study the effects of dose and route of administration on tumor growth and tumor immunity. All mice given 5 x 10^6 viable MOPC-21 tumor cells s.c. or i.p. died, whereas four of five inoculated i.v. survived. As few as 5 x 10^3 tumor cells inoculated i.p. killed all the animals. As few as 10^2 and 10^3 cells given i.v. killed five and six of seven mice, resp., while mice that received 10^5 or more cells i.v. usually survived. Fifteen of 16 mice which had been inoculated with 5 x 10^6 cells i.v. 56 days before challenge with 10^4 cells i.v. survived, while only two of 14 control mice survived. Spleen cells from animals injected i.v. with 10^7 or 10^4 tumor cells seven days prior to removal strongly inhibited or initially enhanced tumor growth, resp. Six of ten mice injected with spleen cells obtained from mice given 10^4 cells i.v. seven days earlier developed tumors, while spleen cells from animals given 10^7 cells i.v. did not develop tumors. I.v. and i.p. injections of tumor cell-stimulated spleen cells were found to suppress tumor growth better than s.c. injections. Immunological enhancement of tumor growth was also demonstrated following sensitization with x-irradiated tumor cells. The results indicate that immunological suppression of tumor cell growth in this system is dependent upon the route of inoculation and the number of cells given. The authors propose that once a tumor has become established, it keeps growing in spite of appreciable immune response against it.

0895 IMMUNE RESPONSE TO A SYNGENEIC MAMMARY ADENOCARCINOMA. III. DEVELOPMENT OF MEMORY AND SUPPRESSOR FUNCTIONS MODULATING CELLULAR CYTOTOXICITY. (Eng.) Kuperman, O. (Immunobiology Res. Center, 1150 University Ave., Univ. Wisconsin, Madison, Wis. 53706); Fortner, G. W.; Lucas, Z. J. *J. Immunol.* 115(5):1282-1287; 1975.

An attempt was made to operationally define cytotoxic, memory, and suppressor cell activities and to examine their sequential changes after inoculation of Fischer rats with a syngeneic mammary carcinoma (13762). In

vitro sensitization of cells from Fischer 344 rats injected 2-10 days earlier with 2×10^7 viable tumor cells always resulted in a higher and earlier lytic response than cells from noninoculated animals. Adoptive transfer of the same *in vivo* primed cells for five days in irradiated syngeneic hosts, and subsequent *in vitro* sensitization still resulted in a higher and earlier cytolytic response. Such cells were defined as "memory" cells for cytotoxicity, were radiosensitive and specific for the immunizing target cell. In contrast to cells from animals inoculated for 3-10 days, cells obtained 11 and 12 days after immunization had a lower response than unprimed cells on *in vitro* sensitization. The anamnestic response could be restored either by culturing 12-day primed cells *in vitro* for two days without antigen or by adoptive transfer for five days into irradiated syngeneic rats. Suppressor cell function was more directly measured by coincubating tumor-primed and unprimed cells on monolayers during *in vitro* sensitization. Cells from animals bearing tumors for 5-10 days always caused an increase in the response of the mixed lymphocyte groups, whereas 11- to 13-day tumor-primed cells always caused a marked decrease in the cytolytic response. These results indicate that cytotoxic cells appear about six days after immunization, reach peak levels two days later, and then decrease rapidly. Memory cells are generated at a faster rate, reach peak levels before maximum cytolytic activity, but are then functionally inhibited from converting into differentiated cytotoxic cells by a new population of suppressor cells which reach peak activity about 12 days after immunization.

0896 RECEPTOR SITES FOR ANTIGEN-ANTIBODY COMPLEXES ON CELLS DERIVED FROM SOLID TUMORS: DETECTION BY MEANS OF ANTIBODY SENSITIZED SHEEP ERYTHROCYTES LABELED WITH TECHNETIUM-99m. (Eng.) Wood, G. W. (Coll. Health Sci. Hosp., Kansas City, Kans.); Gillespie, G. Y.; Barth, R. F. *J. Immunol.* 114(3):950-957; 1975.

Primary cell cultures derived from three murine 3-methylcholanthrene-induced fibrosarcomas, two spontaneous malignant melanomas, a Moloney sarcoma virus-induced tumor in BALB/c mice and the Walker 256 carcinosarcoma of Holtzman rats were found to absorb technetium-99m-labeled, antibody-sensitized sheep erythrocytes (^{99m}Tc EA). After one or two passages *in vitro*, all cells lost their reactivity, but regained it after one passage *in vivo* as determined by radioisotopic quantitation or visual adherence scores. Adherence of ^{99m}Tc EA could be blocked by soluble immune complexes made with ovalbumin and rabbit antibody against it, and by an *E. coli* lipopolysaccharide and mouse antibody against it. Coating of indicator erythrocytes with F(ab')$_2$ fragments of the sensitized sheep erythrocyte antiserum resulted in no adherence. Normal sera from rabbits and mice, immune serum and antigen alone did not block adherence. The results indicate that hemadsorption in these cases required an intact Fc portion of the antibody molecules and that the receptors to which ^{99m}Tc EA binds had a higher affinity for the immune complexes than for antigen or antibody alone.

0897 ASSESSMENT OF REACTIVITIES OF NATURAL ANTIBODIES TO ENDOGENOUS RNA TUMOR VIRUS ENVELOPE ANTIGENS AND VIRUS-INDUCED CELL SURFACE ANTIGENS. (Eng.) Hanna, M. G., Jr. (Oak Ridge Natl. Lab., Tenn.); Ihle, J. N.; Batzing, B. L.; Tennant, R. W.; Schenley, C. K. *Cancer Res.* 35(1):164-171; 1975.

Autogenous humoral immune response of mice to their endogenous leukemia virus (MuLV) was compared to the reactivities of natural antibodies to MuLV envelope antigens and virus-induced cell surface antigens. The mice strains were C57BL/6 retired breeders, C3H/Anf male retired breeders, and male C57BL/6 x C3H/Anf F$_1$ hybrid. Natural reactivity of MuLV envelope antigens was evaluated by radioimmune precipitation assay of intact and disrupted virus and by virus neutralization tests. Natural antibody specificity for MuLV envelope antigens was determined by immunoelectron microscopy and radioimmune precipitation. Antibody reactivity to virus-induced cell surface antigens was evaluated by immunoelectron microscopy and a complement-dependent cytotoxicity test. Various levels of natural antibodies with specificity for endogenous MuLV envelope antigens were found. However, the naturally recognized antigenic determinants of the virus were a consistent aspect of autogenous immunity, i.e., glycoprotein of 68,000-dalton (*gp*68), *gp*48, and 15,000-dalton protein component. High levels of neutralizing antibody against xenotropic BALB: virus-2 were detected in these various normal sera with the focus reduction assay. Only marginal levels of neutralizing activity against Moloney leukemia virus were detected with the XC (cell plaque technique) virus assay. No other cellular antibody was found in these normal sera with the complement dependent cytotoxicity assay. Thus, natural recognition of viral antigenic determinants of endogenous leukemia virus was demonstrated to be a general phenomenon, while natural antigenic recognition of virus-induced cell surface antigens appears to be a more restricted autogenous immune function.

0898 ANTIBODY RESPONSE OF GENETICALLY SUSCEPTIBLE AND RESISTANT CHICKENS TO CELL-FREE AND ATTENUATED JM-V LEUKOSIS STRAIN AND ITS INFLUENCE ON EARLY TYPE II (MAREK'S) LEUKOSIS INFECTION. (Eng.) Shieh, H. K. (Dept. Vet. Anim. Sci., Univ. Massachusetts, Amherst); Sevoian, M. *Poult. Sci.* 54(1):69-77; 1975.

Experiments were carried out to determine various antibody responses to JM strain leukosis virus (JMV) vaccination and exposure. Using JMV-susceptible (JM-P) and JMV-resistant (JM-N) chickens and cell-free vaccines prepared from 18th and 21st embryo passages of JMV, day-old and 3 week-old chickens were inoculated i.p., then bled at day-old, 3-, 6-, and 10-wk of age by the wing vein or by cardiac puncture. Serum samples thus collected were titrated by means of the plaque reduction serum neutralization antibody test (SN) and the indirect fluorescent antibody test. I.p. challenge of the vaccinated chickens was effected by 2000 CLD$_{50}$ of cellular virulent JMV. The SN test confirmed the existence of high levels of neutralizing antibodies

in day-old progeny from dams previously vaccinated with JMV, while little or no neutralizing antibodies were found in offspring of unvaccinated dams. Vaccination of those day old chicks which had low maternal antibody increased the serum antibody, while vaccination of day old chicks which had high maternal antibody decreased the serum antibody. Thus, high levels of maternal antibody had an inhibitory influence on the vaccine antigen. Results of indirect fluorescent antibody tests confirmed this finding since indirect fluorescent antibody titers of vaccinated chicks from vaccinated dams decreased somewhat during a three-week period, while those from unvaccinated dams showed an increase in antibody titer. All vaccinated chicks withstood a challenge dose of 2000 CLD_{50} of cellular JMV, whereas 58% of the unvaccinated controls died from the same challenge. Progeny possessing maternal antibodies inhibited JMV challenge in excess of 3 x $\log_{10}$ times greater than progeny from unvaccinated or turkey herpes virus-vaccinated dams. Also, progeny possessing maternal antibody had significantly fewer oncogenic lesions. The pathogenicity of JMV was markedly decreased by serial passage in chick embryos, as was evidenced by a complete lack of morbidity and mortality of JM-P and JM-N chicks when they were vaccinated with inocula prepared from 18th and 21st chick embryo passages of the JMV strain. The results suggest that the JMV agent is effective in inducing immunity against type II (Marek's) leukosis.

0899 RELEASE OF MITOGENIC FACTOR BY MOUSE LYMPH NODE CELLS STIMULATED WITH PHA *IN VITRO*. INHIBITION OF THIS PHENOMENON BY THE ADDITION OF THYMOCYTES. (Eng.) Jacobsson, H. (Department of Tumor Biology, Karolinska Institutet, S-104 01 Stockholm, Sweden); Blomgren, H. *J. Immunol.* 114(5): 1623-1630; 1975.

Thymidine uptakes of CBA mouse lymph node cells and thymocytes in response to phytomitogen lectins were investigated *in vitro*. Varying numbers of lymph node cells and thymocytes from normal or cortisone (i.p. 150 mg/kg body weight) treated animals were cultured together or separately and incubated with various mitogens. Each culture received 0.02 ml RMPI containing 1.0 µCi of tritiated thymidine (^{3}H-TdR, specific activity 5 Ci/mM). Mixtures of lymph node cells and thymocytes yielded higher ^{3}H-TdR incorporations than expected when exposed to 7.5 mg/ml concanavalin A (Con A) or a 2% solution of pokeweed mitogen (PWM), but lower incorporations were achieved with 1.0% phytohemagglutinin (PHA) as a stimulant. The observed enhancement of the response could be explained by factors which are stimulatory for thymocytes released by the lymph node cells. However, such mitogenic factors (MF) were released not only by Con A- and PWM-exposed cells but also by PHA-exposed lymph node cells. Further experiments showed that the admixture of thymocytes inhibited the release of MF by lymph node cells exposed to PHA but not to Con A or PWM. Since medullary thymocytes did not exhibit any inhibitory activity, it is likely that cortical thymocytes were responsible for this effect. MF was efficiently produced by lymph node cells cultured with PHA in thymocyte-conditioned medium and the MF activity was only marginally decreased by absorption with thymocytes. The results strongly indicate that the increment of stimulation of thymocytes is due to factors released by the highly responsive cells upon mitogen stimulation which stimulate or potentiate the response of thymocytes.

0900 GENETIC REGULATION OF THE ANTIBODY RESPONSE TO H-2D^b ALLOANTIGENS IN MICE. I. DIFFERENCES IN ACTIVATION OF HELPER T CELLS IN C57BL/10 AND BALB/c CONGENIC STRAINS. (Eng.) Wernet, D. (Albert Einstein Coll. Med., Bronx, N.Y.); Lilly, F. *J. Exp. Med.* 141(3):573-583; 1975.

C57BL/10, B10 and A.BY mice were used in order to determine if the altered antibody response of C57BL/10 mice was associated within or outside of the H-2 complex. Lymphocyte-mediated cytolysis was measured using peritoneal exudate cells of immunized mice and target cells of C57BL leukemia or phytohemagglutinin-induced blast cells. Normal T-cell-mediated cytotoxicity to H-2D^b tumor cells was seen in B10.A(5R) mice immunized with C57BL/10 spleen cells, but no antibody response to H-2D^b alloantigens was seen. An IgG response was seen in B10.A(5R) mice immunized with A.BY or B10.A(2R) cells. The B10.A(2R) cells differ from B10.A(5R) cells at H-2D^b, H-2K^k and H-2I^k cell surface antigens, whereas the A.BY differ at H-2D^b and non-H-2 cell surface antigens. C57BL/10 mice immunized with congenic H-28 cells made only IgM antibodies, whereas BALB/c mice made IgG antibodies. T-helper cells failed to switch from IgM to IgG when the only differences on the immunizing cells were the H-2D^b alloantigens. The results indicate that T-cell helper function is regulated by genes separate from H-2, and that T-cell helper function is regulated by different genes from those that regulate T-cell mediated cytotoxicity.

0901 AGGLUTININS OF FROG EGGS: A NEW CLASS OF PROTEINS CAUSING PREFERENTIAL AGGLUTINATION OF TUMOR CELLS. (Eng.) Kawauchi, H. (Tohoku Coll. Pharm. Sci., Sendai, Japan); Sakakibara, F.; Watanabe, K. *Experientia* 31(3):364-365; 1975.

Eggs from seven species of frogs were homogenized and centrifuged; the supernatant was dialyzed, lyophilized, and then chromatographed on a Sephadex G-75 column to test the agglutination activity of the product towards normal and tumor cells. Distinct agglutination activity against 2 ascites hepatoma cell types (AH109A and AH130), polyoma-transformed NIL and BHK cells and SV40 virus-transformed 3T3 cells was found in preparations from *Rana japonica* Guenther and *Rana nigromaculata nigromaculata* Hallowell eggs. *Rana japonica* agglutinins had no effect on rat and human RBC while *Rana nigromaculata nigromaculata* agglutinins had activity against blood group A RBC. Normal cultured cells could be agglutinated by the egg preparations active against transformed cells, but 25-200 times the concentration of agglutinin was required. Monosaccharides and oligosaccharides did not inhibit the agglu-

tination but a ganglioside fraction of human RBC membrane did. Cellulose acetate electrophoresis showed the agglutinin fraction to be composed of basic proteins only. The results indicate that eggs obtained from certain species of frogs contain a protein or proteins capable of selective agglutination of tumor cells.

0902 *IN VITRO* AND *IN VIVO* ALTERATIONS OF LONG-TERM MURINE PLASMACYTOMA CULTURES. (Eng.) Sorenson, G. D. (Dartmouth Med. Sch., Hanover, N.H.); Pettengill, O. S. *Arch. Intern. Med.* 135(1):114-118; 1975.

Two sublines (L-1, L-2) of a mouse myeloma cell line (SLU-5) derived from MOPC-21 tumor cells were cultured and inoculated into irradiated mice to determine the tumorigenicity and immunogenicity of these lines. Mice, 8-10 weeks old, were x-irradiated with a total dose of 400 rads in a 20 x 20 cm field, then injected 12 hrs later with 10^7 SLU-5(L-1) or SLU-5 (L-2) cells. BALB/c mice were immunized i.p. with 10^6 L-1, L-2 or MOPC-21 cells weekly for three weeks. On the fourth week, they were challenged s.c. with 2.5 x 10^4 MOPC-21 cells. L-1 cells were tumorigenic in 100% of irradiated mice and lethal in 20%, compared to 0% lethality and tumorigenicity in ten nonirradiated controls; L-2 cells were tumorigenic in all controls and treated mice, but lethal in only 20% of controls and 80% of irradiated mice. L-2 cells were more easily damaged by physical manipulation than L-1, while L-1 had increased cell coat staining by ruthenium red, and produced 10.2 μg globulin/10^5 cells, compared to 19.5 μg globulin/10^5 cells of the L-2 line. Immunization with L-1 and L-2 resulted in decreased tumor production by MOPC-21 (70% tumor incidence in 57 mice and 84% in 51 mice, resp.) compared to immunization with MOPC-21 (100% incidence in 16 mice) and controls (100% in 24 mice). L-1 cells were more immunogenic than L-2 cells which are more immunogenic than MOPC-21 cells, while the order of tumorigenicity was the reverse. The authors speculate that there are four different types of cells within these cell lines (type 1 being highly oncogenic and nonimmunogenic, while type 4 is highly immunogenic and nononcogenic) and that the differences in the sublines result from selection and predominance of these types (L-1 being predominantly type 3 and 4 cells, L-2 being predominately type 1 and 2 cells).

0903 EARLY ONSET OF SERUM BLOCKING IN A MURINE MELANOMA MODEL. (Eng.) Bray, A. E. (Dept. Surgery, Univ. N.S.W., Kensington 2033, N.S.W., Australia); Holt, P. G.; Roberts, L. M.; Keast, D. *Int. J. Cancer* 16(4):607-615; 1975.

The early onset of serum blocking of host cell-mediated immunity was investigated in a transplantable murine melanoma model employing two *in vitro* assays. One of these was based on the Takasugi and Klein cytotoxicity test; the other was a new test based on the inhibition of adherence of WBC to a plastic surface in the presence of tumor antigen. All mice used were females of the C57Bl/6J strain, aged six to eight wks. Both tests yielded similar results, and the adherence inhibition test was more sensitive. Blocking could be detected as early as 1 hr after the inoculation of 1 x 10^7 melanoma cells. Early onset of serum blocking was also demonstrable if homogenized or tissue-cultured tumor cells were inoculated. Serum blocking under these circumstances was initially transient, declining at 24 to 48 hr, but reappearing by the 4th day after inoculation. Furthermore, a functional immune system was not required for blocking factor development in this system, as evidenced by the onset of a similar pattern of blocking activity in heavily irradiated animals.

0904 DIFFERENTIAL CYTOTOXICITY OF TUMORIGENIC AND NONTUMORIGENIC STRAIN-2 GUINEA PIG CELLS AS MEDIATED BY SYNGENEIC PHYTOHEMAGGLUTININ-STIMULATED PERITONEAL EXUDATE CELLS. (Eng.) Zwilling, B. S. (Natl. Cancer Inst., Bethesda, Md.); Meltzer, M. S.; Evans, C. H. *J. Natl. Cancer Inst.* 54(3):743-747; 1975.

Male Sewall Wright strain-2 guinea pigs were immunized with intradermal injections of Phipps strain *M. bovis* strain BCG to determine if peritoneal exudate cells (PEC) obtained from them would show greater toxicity towards tumorigenic cultured cells than towards nontumorigenic cells. The cytotoxicity of unstimulated and purified protein derivative of tuberculin (PDD)- and phytohemagglutinin (PHA)-stimulated PEC were determined by the release of ^{3}H-thymidine from previously labeled (1) morphologically transformed tumorigenic target cells, (2) nontransformed nontumorigenic target cells in culture for long or short periods and (3) transformed nontumorigenic target cells. Tumorigenic and nontumorigenic cells were destroyed by PPD-stimulated BCG-immune PEC, but a greater release of ^{3}H-thymidine was observed from tumorigenic cells. PHA-stimulated BCG-immune PEC produced the same pattern of cytotoxicity towards target cells. PHA-stimulated PEC from nonimmune animals also produced this cytotoxicity pattern. Dose-effect studies revealed BCG-immune PEC to show greatest differential toxicity between tumorigenic and nontumorigenic target cells at a PHA concentration of 50 μg/ml, and PEC numbers of 0.5 x 10^6 to 1.0 x 10^6 cells/ml. The results indicate that measurement of PEC cytotoxicity towards tumorigenic and nontumorigenic target cells may be useful in categorizing culture cells of unknown tumorigenicity.

0905 NATURAL CYTOTOXIC REACTIVITY OF MOUSE LYMPHOID CELLS AGAINST SYNGENEIC AND ALLOGENEIC TUMORS. I. DISTRIBUTION OF REACTIVITY AND SPECIFICITY. (Eng.) Herberman, R. B. (Natl. Cancer Inst., Building 8, Room 118, Bethesda, Md. 20014); Nunn, M. E.; Lavrin, D. H.. *Int. J. Cancer* 16(2):216-229; 1975.

Lymphoid cells from normal mice of a variety of inbred strains were tested for cell-mediated cytotoxicity against syngeneic and allogeneic tumor cells in a ^{31}Cr release assay. Most studies were performed with Rauscher virus-induced RBL-5 target cells. C57Bl/6N mice had significant but low levels of cyto-

toxicity against syngeneic RBL-5 cells, whereas spleen cells from $B6C3F_1$ were very reactive, followed by cells from CBA mice. With the exception of A/Jax mice, who had little or no reactivity, intermediate levels of reactivity were seen with the other strains tested. Very high reactivity was also seen with effector cells from athymic nude mice, which was consistent with other evidence that the reactivity was not T-cell-dependent. Target cells susceptible to lysis included other tumors induced by oncogenic type-C viruses and also tumors induced by other means and expressing endogenous type-C viruses. The levels of natural reactivity were influenced by age, with highest cytotoxicity produced by cells from 5-8-wk-old mice. Lymph-node cells, spleen cells, peritoneal exudate cells, and peripheral blood lymphocytes all had cytotoxic reactivity. The specificity of the reactions was analyzed in detail by an inhibition assay. Evidence was obtained for natural reactivity against several different antigens, each apparently associated with expression of murine endogenous type-C viruses.

0906 TREATMENT OF RUNTING SYNDROME AND PREVENTION OF PRIMARY LYMPHOMAS IN FRIEND VIRUS-TOLERANT RATS. (Eng.) Takeichi, N. (Hokkaido Univ. Sch. Med., Sapporo, Japan); Kuzumaki, N.; Kobayashi, H. *Cancer Res.* 35(3):729-733; 1975.

Male and female WKA/Mk rats were injected with Friend's virus (FV) or Friend lymphoma (WFT-13) in order to study the pathological mechanism of the runting syndrome and attempt to cure it. Rats made tolerant to FV by high dose injections of FV within 48 hr of birth succumbed to the runting syndrome following inoculation with spleen and lymph node cells from rats which had been immunized with WFT-13 cells by 4-5 s.c. injections. All rats inoculated with normal spleen and lymph node cells or normal thymus cells along with immune spleen and lymph node cells died of the runting syndrome, but survival was prolonged by normal thymus cells. Twelve of 15 rats given normal bone marrow cells along with immune spleen and lymph node cells survived, but showed evidence of the runting syndrome. WFT-13 cell injections two weeks later resulted in three deaths due to runting but the remaining nine survived more than 300 days without evidence of lymphoma development. Trypan blue cytotoxicity assays indicated that the FV-infected cells were distributed in thymus, spleen and bone marrow and were killed by inoculations with immune cells. The results indicate that inoculation with syngeneic bone marrow cells was successful in preventing the runting death, and also in preventing primary lymphoma development. The authors suggest that this may be due to the replacement of FV-infected cells by new bone marrow cells.

0907 REACTIONS OF MURINE MYELOMA CELLS WITH INFECTIOUS MONONUCLEOSIS SERA. (Eng.) Yoshida, H. (Sch. Med., State Univ. New York, Buffalo, N.Y.); Kano, K.; Milgrom, F. *J. Immunol.* 114(5): 1449-1453; 1975.

Reactions of murine myeloma cells with infectious mononucleosis (IM) sera were studied by means of cytolysis in agarose gel. Sera of patients with various diseases were tested against murine plasmacytoma line MOPC-104E employing preincubation at 20 C. Thirty of 75 sera from patients with IM and 21 of 35 sera from patients with various viral infections had cytolytic titers of 320 or more. A comparison of titers of IM sera obtained from cytolysis of MOPC-104E cells and from lysis of bovine RBC showed there was no apparent correlation between titers of these two types of antibodies. Three types of antibodies acting upon the myeloma cells were identified serologically on the basis of absorption experiments with the bovine RBC, θ-positive murine lymphoma cells and guinea pig kidney cells. Antibodies of the first group could be absorbed with none of these antigens, antibodies of the second group could be absorbed only with lymphoma cells, and antibodies of the third group could be absorbed with any of the three antigens. Indirect membrane immunofluorescence tests were performed on the myeloma cells as well as θ-positive lymphoma EL-4 cells using fluorescein-isothiocyanate-labeled anti-IgM serum. Positive reactions were observed with 30-40% of the myeloma cells by using IM serum absorbed with bovine RBC and with over 95% of the EL-4 cells by using IM serum absorbed with the myeloma cells. When anti-IgM conjugate was replaced by anti-IgG conjugate all results were negative. This was in accord with the well known IgM nature of Paul-Bunnell antibodies and showed that the antibodies combining with MOPC-104E belong also to this class of immunoglobulins. The results show that 40% of IM sera contain lytic antibodies for murine IgM-producing myeloma cells MOPC-104E. These antibodies are independent from any other antibodies previously shown in IM sera.

0908 SUPPRESSOR CELLS IN TUMOR-BEARING MICE CAPABLE OF NONSPECIFIC BLOCKING OF *IN VITRO* IMMUNIZATION AGAINST TRANSPLANT ANTIGENS. (Eng.) Eggers, A. E. (Natl. Cancer Inst., Bethesda, Md.); Wunderlich, J. R. *J. Immunol.* 114(5):1554-1556; 1975.

Nylon-adherent non-θ-bearing cells were identified in tumor-bearing mice which were capable of nonspecifically suppressing the ability of lymphocytes from normal mice to be immunized *in vitro* against transplant alloantigens. A methylcholanthrene (MCA)-induced tumor was produced in C57BL/6N (B6) mice by injecting them with 5 μg/MCA. Spleen cells from the tumor-bearing mice developed less cytotoxic activity when cultured *in vitro* for five days with allogeneic irradiated spleen cells than did spleen cells from normal mice. The hyporesponsiveness of spleen cells from tumor-bearing mice was apparent as soon as one week after the appearance of palpable tumors and persisted to the time of death. Spleen cells from normal and tumor-bearing donors were fractionated by treatment with anti-θ and nylon column passage and remixed before sensitization. All cells, when mixed with anti-θ-treated cells from normal mice, gave a response similar to that of unseparated normal spleen cells; the same nylon-passaged cells, when mixed with anti-θ-treated cells from tumor-bearing mice, gave a reduced response

similar to that of unseparated spleen cells from tumor-bearing mice. Two possibilities are suggested by the results: (1) a population of nylon-adherent non-θ-bearing cells necessary for the development and expression of T cell function is deficient in the tumor-bearing mice; or (2) that there is a population of nylon-adherent non-θ-bearing suppressor cells in the tumor hosts which can inhibit the response of either normal or tumor host T cells.

0909 SEROLOGICAL ANALYSIS OF HUMAN DEOXYRIBONUCLEIC ACID POLYMERASES: PREPARATION AND PROPERTIES OF ANTISERUM TO DEOXYRIBONUCLEIC ACID POLYMERASE I FROM HUMAN LYMPHOID CELLS. (Eng.) Smith, R. G. (Natl. Cancer Inst., Bethesda, Md.); Abrell, J. W.; Lewis, B. J.; Gallo, R. C. *J. Biol. Chem.* 250(5):1702-1709; 1975.

A method for the preparation of an antiserum to a purified DNA polymerase I (6 to 8 S), obtained from the human lymphoid cell line RPMI 1788, was developed to help define the relationship between this DNA polymerase and that isolated from mammalian mitochondria and cell cytoplasm. One μg of the antipolymerase immunoglobulin G (prepared in rats) was found to inhibit 60% of the activity of 54 ng of DNA polymerase I when incubation was maintained for 48 hours. The antigen-antibody complex was precipitated with goat anti-rat immunoglobulin G and the remaining activity of the supernatant was measured. This procedure revealed that 2.2 μg of the antipolymerase antibody bound 33 ng of the DNA polymerase I. Little, if any, antigenic relationship between DNA polymerase I and DNA polymerase II and five viral DNA polymerases was seen using the neutralization assay and the immunoprecipitation test. In order to inhibit polymerase activity in these preparations, concentrations 50 times higher than those necessary for DNA polymerase I inhibition were needed. Likewise, DNA polymerases obtained from leukocytes from two patients with acute myelogenous leukemia, and from rhesus monkey placenta were not inhibited by concentrations of the antibody which inhibited 76% of the activity of DNA polymerase I. It is proposed that this rat antiserum may be useful for testing other DNA polymerase preparations for contamination with DNA polymerase I.

0910 ANTINUCLEAR REACTIVITY OF SERA IN PATIENTS WITH LEUKEMIA AND OTHER NEOPLASTIC DISEASES. (Eng.) Steiner, M. (Dept. Tumor Biology, Karolinska Institutet, S-104 01 Stockholm 60, Sweden); Klein, E.; Klein, G. *Clin. Immunol. Immunopathol.* 4(3):374-381; 1975.

An extended study was made to elucidate the nature of the previously reported anticomplement immunofluorescence (ACIF) reaction between sera of acute myelogenous leukemia (AML) patients and nuclei of fixed myeloblasts. Four different groups of target cells were used, the first consisting of lymphocytes from normal peripheral blood (Ly), myeloblasts from AML patients, and leukemia cells from chronic myelogenous leukemia (CML) and chronic lymphatic leukemia (CLL) patients; the second consisting of PHA-transformed lymphocytes; the third, a lymphoblastoid T-cell line (Molt-4) and a myeloma line (Simpsson); the fourth, cultured human embryonic lung fibroblasts. The sera which were tested originated from five categories, the first representing lymphoproliferative diseases, namely, AML, acute lymphatic leukemia (ALL), CML, CLL, African Burkitt's lymphoma (BL), American Burkitt's lymphoma (ABL), Hodgkin's disease (HD), polycythemia vera (PCV), myeloma (MY), and infectious mononucleosis (IM); the second, connective tissue diseases rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and unspecified autoimmune diseases (AD); the third, cancer of the breast, nasopharynx, and other locations; the fourth, non-cancer normal controls; the fifth, suspected preleukemic conditions. The test consisted of preparation of smears of buffy coat or cultured cells in 0.95% Na citrate, rinsing with 0.075 M KCl, treatment with test serum and with human C', staining with fluorescein-conjugated goat anti-human B_1CB_1A-globulin, and microscopic examination for characteristic speckled pattern. AML blasts, PHA-transformed lymphocytes, established EBV genome-free myeloma or lymphoma-derived cell lines, and cultured embryonic lung fibroblasts gave positive reactions, whereas normal lymphocytes, CML cells, and CLL cells were negative. All target cells, however, reacted with SLE sera. It thus appeared that the particular anti-nuclear antigen (ANA) being studied was undetectable in cells which do not multiply and that its expression may be related to a change in the state of the chromatin in the proliferating cells. Of the sera tested, positive results were obtained with 73% of 137 AML specimens, 40% of 37 ALL, 26% of 35 CML, 14% of 44 CLL, 13% of 46 BL, 28% of 50 ABL, 11% of 21 PCV, 28% of 25 MY, 18% of 54 Ca, 7% of 27 HD, 10% of 10 IM, 9% of 42 normal pregnant, and 1.3% of 77 healthy. Among four serum-positive suspected preleukemic patients, two developed AML within six months. Since leukemic sera were previously reported to be negative, as a rule, for ANA, it appeared that the ACIF test may amplify reactions which are too weak for detection by direct or indirect immunofluorescence.

0911 COMPLEMENT SENSITIVITY OF SOMATIC HYBRIDS OF A COMPLEMENT-RESISTANT MURINE LEUKEMIA CELL LINE. (Eng.) Liang, W. (La Rabida Children's Hosp. and Res. Center, E. 65th St. at Lake Michigan, Chicago, Ill. 60649); Cohen*, E. P. *J. Natl. Cancer Inst.* 55(2):309-317; 1975.

Since the resistance of murine leukemia line RADA-1 ($H-2^a$) cells to cytotoxic action of guinea pig complement (GPC) and antiserum to thymus-leukemia (TL) antigen appeared to be related to the low density of TL antigen on the cells, further investigation of the mechanism of complement (C) resistance was carried out using hybrids of RADA-1 and $LM(TK)^-$ ($H-2^k$) a thymidine kinase-deficient mutant line of mouse L cells. RADA-1 was maintained by serial transfer in histocompatible recipients; $LM(TK)^-$, as a cell culture. Cell fusion was accomplished with the aid of β-propiolactone-inactivated Sendai virus. Clones of hybrid cells were isolated with a platinum loop and propagated in hypoxanthine-aminopterin-thymidine medium. Sensitivity of target cells was determined by a C-mediated microcytotoxicity test

using antisera specific for various TL, Thy, and H-2 antigens. Expression of TL antigens was determined by mixed hemagglutination and immunofluorescence procedures. Density of TL antigens on cells was determined by immunoprecipitation of Nonidet 40-solubilized hybrid and parental cells, using TL antiserum and rabbit anti-mouse IgG. The cells were labeled with tritiated fucose or ^{125}I before they were extracted. The immunoprecipitates were analyzed further by resolubilization and electrophoresis in SDS-polyacrylamide gel. The hybrid cells expressed the H-2^a and H-2^k antigens of the parents, shared their chromosomes, and were larger than either parent. The hybrids formed TL 1, 2, 3 antigens as determined by immunofluorescence, mixed hemagglutination, direct isolation of TL antigens, and capacity of the cells to reduce known titers of TL antiserum. The hybrids lost the capacity to resist lysis by TL antiserum and GPC. They were susceptible to cytotoxic effects of GPC and antisera to TL 1, 2, 3, TL 2, or TL 1, 3, even though the density of TL antigens associated with these cells was only 25% of the density of the resistant RADA-1 parental cells. These results indicated, therefore, that the mechanism of resistance to C-mediated lysis was genetically separable from the expression of TL antigens by the cells and that the susceptibility of the cells to the cytotoxic effects of antiserum was related only in part to the density of TL antigens expressed by the cells.

0912 DETECTION OF CELL-BOUND IMMUNOGLOBULINS BY A RADIOISOTOPIC MICRO-MIXED HEMADSORPTION REACTION WITH TECHNETIUM-99m-LABELED ERYTHROCYTES. (Eng.) Wood, G. W. (Coll. Health Sci. Hosp., Kansas City, Kan.); Barth, R. F. *J. Immunol.* 114(3):944-949; 1975.

A radioisotopic modification of the mixed hemadsorption (MHA) reaction is reported which had two times the sensitivity of visual assessment, 100 to 200 times that of ^{125}I-mixed antiglobulin reactions and 500 to 1000 times that of indirect immunofluorescence techniques. The modification involves the prelabeling of sheep erythrocytes with technetium-99m (^{99m}Tc), which is a metastable gamma-emitter with a short half life and high specific activity. The method was used successfully to measure high titers of antibody activity in species-specific xenoantisera and mono- and polyspecific alloantisera. The species of origin of four human tumor cell lines and two murine cell lines was successfully predicted by this assay system. The results indicate that the ^{99m}Tc modification of the MHA reaction is a sensitive, quantitative test providing accurate identification of membrane reactive antibodies and cell surface antigens.

0913 CONCURRENT MONOCLONAL IgM AND IgA PROTEINS IN LYMPHOCYTIC LYMPHOMA. (Eng.) Tormey, D. C. (Natl. Cancer Inst., Bethesda, Md. 20014); Ellison, R. R.; Hossfeld, D. K. *Cancer* 36(4):1321-1326; 1975.

A case of a 78-yr-old man with lymphocytic lymphoma was studied with respect to levels of serum immunoglobulins. Serum protein electrophoresis revealed an abnormal β-globulin peak containing 4.8 g/100 ml of the total protein of 8.7 g/100 ml. Immunoelectrophoresis with specific antisera showed abnormally high amounts of κ-IgA and κ-IgM in the serum. Quantitative radial immunodiffusion revealed 23.7 mg/ml IgA, 14.2 mg/ml IgM, and 9.0 mg/ml IgG. Ultracentrifugation disclosed 16.7S, 12.8S, 11.0S, and 9.0S peaks. The 16.7S peak was compatible with IgM; the other peaks were compatible with IgA polymers. There was 290 mg/ml urinary protein, with the following cellulose acetate electrophoretic distribution: albumin 2%, α_1-globulin 0%, α_2-globulin 7%, β-globulin 13%, and λ-globulin 78%. The patient's peripheral lymphocytes were stimulated *in vitro* with phytohemagglutinin as revealed by uptake of ^{14}C-leucine. Immunofluorescent staining reactions of bone marrow specimens indicated that the IgA and IgM were produced in different cells. Previously reported immunochemical analysis of the individually specific determinants of the two immunoglobulins, together with results of cytogenetic analysis of cultures of peripheral lymphocytes of this patient, suggests that the different cells involved were derived from a common precursor cell.

0914 ELEVATED IgA IN CARCINOMA OF THE NASOPHARYNX. (Eng.) Wara, W. M. (Dep. Radiol., Univ. California, San Francisco); Wara, D. W.; Phillips, T. L.; Ammann, A. J. *Cancer* 35(5):1313-1315; 1975.

Fourteen nasopharyngeal carcinoma patients were studied to determine if a specific pattern of immunologic aberration existed. Serum immunoglobulins were measured by single radial diffusion and IgA levels were elevated (range 300-1000 mg/100 ml; mean 549 mg/100 ml) in all of the patients. Serum IgM and IgG levels were all normal. Antibody titers to hexavalent pneumococcal polysaccharide and keyhole limpet hemocyanin (KLH) were measured by indirect hemagglutination and were elevated 2-fold in nine of 11 patients immunized with pneumococcal polysaccharide, but not in four of seven patients immunized with KLH. Cell-mediated immunity was decreased in seven of 11 patients when determined by T-cell rosette formation and *in vitro* stimulation of ^{14}C-thymidine incorporation by phytohemagglutinin. The results indicate that elevated serum IgA and depressed cell-mediated immunity may be characteristic findings in patients with nasopharyngeal carcinoma. The authors suggest that elevated serum IgA levels may be useful as an early marker of nasopharyngeal carcinoma in high-risk populations.

0915 ANTI-TUMOR ANTIBODIES IN NORMAL MOUSE SERA. (Eng.) Martin, S. E. (Zool. Dept. Univ. Coll., London, England); Martin, W. J. *Int. J. Cancer* 15(4):658-664; 1975.

Normal mouse sera were tested for antibody activity against several tumor-cell types. Using congenitally athymic (nude) mice and CBA mice of 3-6-mo of age,

sera obtained from the tail vein were either stored in aliquots or tested directly in a trypan-blue dye cytotoxicity assay. In testing for complement-dependent cytotoxic antibody activity, pooled syngeneic sera regularly caused significant complement-dependent lysis of lymphosarcoma γG2LS, teratoma-derived cell lines NB1 and TerB liver cell lines E-10 and A-10, lung tumor 85-72, neuroblastoma C1300, and leukemias EL-4 and L1210. Testing of individual sera indicated that the various mouse strains differed in their capacity to lyse the target cells, and that the degree of lysis of one target cell by a given serum did not correlate with the degree of lysis of other target cells. Sera obtained from nude mice were cytotoxic for γG2LS, TerB, EL-4, C1300, and L1210 tumor cells. Heat, 2-mercaptoethanol treatment, or incubation with goat anti-mouse immunoglobulin M (IgM) antibody reduced the cytotoxicity of sera from normal C3H mice against target cells. In cross-absorption studies, it was found that NB1 cells absorbed anti-NB1 cytotoxic activity without significantly reducing cytotoxic activity against 85-72 or E-10 cells. Similarly, 85-72 and E-10 cells selectively absorbed cytotoxic activity against 85-72 and E-10 cells, respectively. Additional absorption studies revealed that absorption with normal C3H tissues did not cause significant reduction of cytotoxicity against C1300, 85-72 or CMT-64 lines of cells, although some reduction of anti-NB1 activity was caused by absorption with brain and kidney tissues. The data indicated that in the presence of adequate amounts of rabbit complement, normal mouse serum is cytotoxic for a variety of syngeneic and allogeneic tumor-cell lines. Susceptibility to 2-mercaptoethanol attributed the cytotoxicity to IgM antibodies, while the cross-absorption studies indicated the expression of serologically distinct antigens.

0916 ANTIBODY SYNTHESIS IN SYNCHRONIZED MOUSE SPLEEN CELLS DURING THE SECONDARY RESPONSE TO SHEEP ERYTHROCYTES *IN VITRO*. (Eng.) Thomas, D. W. (Univ. Colorado Med. Sch., Denver); Roberts, W. K.; Talmage, D. W. *J. Immunol.* 114(1):343-347; 1975.

Immunoglobulin synthesis by mouse spleen cells during the secondary response to sheep RBC *in vitro* was examined in cells synchronized by methotrexate and adenosine treatment. Antibody synthesis by the synchronized cells was discontinuous through the cell cycle and highest in the early S phase. Previous data had indicated that antibody secretion, as measured by plaque production to sheep RBC, was also greatest in the early S phase. Taken together, these results suggest little difference in the expression of antibody synthesis and secretion through the cell cycle. Actinomycin D was employed in several experiments in an attempt to determine whether the variation in antibody synthesis was regulated by the transcription of messenger RNA for immunoglobulin. Lower actinomycin concentrations (0.05 μg/ml), which reportedly permit messenger but not ribosomal RNA synthesis, rapidly reduced immunoglobulin and total protein synthesis; higher concentrations (1 μg/ml) had similar effects. These results indicated that actinomycin was not suitable for determining the stability of messenger RNA in mouse spleen cells. Although it was not, therefore, possible to determine the reasons for fluctuations in antibody synthesis during the cell cycle, the following explanations may hold: the level of immunoglobulin and protein synthesis is regulated by the amount of available mRNA, which in turn may be controlled by the extent of transcription or post-transcriptional modification of the mRNA; protein and antibody synthesis are controlled by factors which allow more efficient translation of immunoglobulin mRNA; or, in the case of antibody, post-translational modifications in the immunoglobulin heavy or light chains may limit the extent of association to form functional antibody molecules.

0917 ANOMALOUS REACTIONS OF MOUSE ALLOANTISERA WITH CULTURED TUMOR CELLS. II. CYTOTOXICITY IS CAUSED BY ANTIBODIES TO LEUKEMIA VIRUSES. (Eng.) Nowinski, R. C. (Fred Hutchinson Cancer Res. Center, 1124 Columbia St., Seattle, Wash. 98104); Klein, P. A. *J. Immunol.* 115(5):1261-1268; 1975.

Anomalous anti-tumor antibody was previously found in mouse alloantisera and in the sera of aged nonimmunized mice of the strains used to produce the alloantisera. In this study the possibility was tested of a relationship between these antibodies and those against murine leukemia virus (MuLV) occurring naturally in mice. The cultured cell lines included B10 and B6 sarcomas induced in C57BL/10 and C57BL/6 mice with 20-methylcholanthrene (MCA) and various spontaneous, chemically-induced, Gross virus-induced, or radiation-induced leukemias of mice and rats. The alloantisera were prepared in mice against H-2 region antigens. Complement-dependent cytotoxicity assays were based on ^{51}Cr-release from labeled target cells. Radioimmune precipitation assays were used for the detection of mouse antibody against intact MuLV. To identify the p15 and gp70 viral antigens with which allogeneic and normal sera reacted, the various sera were incubated with ND40 detergent-disrupted MuLV, and the resultant immune precipitates were examined by polyacrylamide gel electrophoresis. Certain alloantisera prepared against H-2 antigens were cytotoxic for the different murine sarcoma and leukemia cells in culture. This was shown to be the result of the action of antibody directed against the p15 and gp70 envelope proteins of MuLV, which were present on the surface of the tumor target cells. Sera from aged unimmunized mice also contained antibodies against MuLV proteins p15 and gp70 that were cytotoxic for the target sarcoma and leukemia cells, indicating that these antibodies occurred naturally in the mice. The presence of these antibodies indicates that caution should be used during the typing of murine sarcomas or leukemias for cell surface antigens, since mouse antisera may yield cytotoxicity based on anti-MuLV specificities rather than on anticipated antigens.

0918 EFFECT OF INTERFERON ON INDUCTION OF S ANTIGEN BY POLYOMA VIRUS IN BHK 21 CELLS. (Eng.) Birg, F. (INSERM Res. Unit, Marseille, France); Meyer, G. *J. Gen. Virol.* 26(2):201-204; 1975.

Induction of S antigen and the effects of interferon were studied in the polyoma virus-induced abortive transformation of hamster cells. Cultured BHK 21 hamster cells were infected with 100 plaque-forming units/cell of polyoma virus which was adsorbed for three hr at 37 C. Two interferon preparations were used: one had been induced by vaccinia virus in hamster embryo fibroblasts; the other had been induced in mouse MSVIC cells by infection with irradiated Newcastle Disease virus. The second interferon preparation was dialyzed and filtrated along with a supernatant fluid from MSVI mouse cells which acted as a control. The cells were either treated with interferon for 12 hr, infected, and then re-fed medium containing interferon, or treated with interferon following virus adsorption. Cells were harvested up to 36 hr after infection and tested for the presence of S antigen using immunofluorescent staining. Antisera were obtained from protected hamsters which had rejected a polyoma-induced tumor graft. Rabbit anti-hamster gamma globulin serum labeled with fluorescein isothiocyanate was adsorbed on hamster and mouse liver powder before use. The hamster interferon gave a 50% reduction in the number of S antigen positive cells at 18 hr after infection. Mouse interferon preparation gave the same results. Induction of S antigen showed the same sensitivity to interferon as induction of other virus functions such as T antigen or stimulation of cellular DNA synthesis. In abortive transformation, the appearance of S antigen could have been related to the expression of a virus gene product.

0919 PRODUCTION OF INTERFERON BY IMMUNE LYMPHOCYTES EXPOSED TO HERPES SIMPLEX VIRUS-ANTIBODY COMPLEXES. (Eng.) Fujibayashi, T. (Tokyo Medical and Dental Univ., 5-45, 1 Chrome, Yushima, Bunkyo-Ku, Tokyo 113, Japan); Hooks, J. J.; Notkins, L. *J. Immunol.* 115(5):1191-1193; 1975.

An investigation was initiated to determine whether immune lymphocytes exposed to herpes simplex virus (HSV)-anti-HSV complexes are capable of inducing interferon. HSV, Type 1, was grown in primary rabbit kidney cells (PRK), and was partially purified. Antiserum to HSV was prepared in rabbits. The serum was separated into IgG and IgM fractions by gel-filtration on Sephadex G-200. HSV neutralization tests with sera and fractionated immunoglobulins were carried out by a plaque reduction method. Induction of interferon in lymphocyte suspensions was accomplished with UV-inactivated HSV and with virus-antibody complexes; interferon activity was assayed by reduction of vesicular stomatitis virus plaque formation on PRK cells. Stimulation of lymphocytes by these antigen or antigen-antibody complexes was measured by incorporation of tritiated thymidine. Splenic WBC from rabbits immunized with HSV incorporated significant amounts of tritiated thymidine and produced interferon after *in vitro* incubation with HSV antigens. In contrast, splenic WBC from nonimmune rabbits gave negative results. Antigen-antibody complexes also were capable, however, of stimulating immune WBC to incorporate tritiated thymidine and produce interferon. It appears that the host has an effective way of protecting cells from destruction by neutralizing infectious virus, while at the same time allowing virus-antibody complexes to stimulate immune lymphocytes to produce interferon.

0920 ENHANCEMENT OF INTERFERON PRODUCTION BY POLY(rI)·POLY(rC) IN MOUSE CELL CULTURES BY ASCORBIC ACID. (Eng.) Siegel, B. V. (Univ. Oregon Med. Sch., Portland). *Nature* 254(5500):531-532; 1975.

The effect of L-ascorbic acid on interferon synthesis in mouse cell cultures was examined in order to determine if previously reported ascorbic acid stimulation of interferon synthesis *in vivo* occurred *in vitro*. Transformed mouse L cells and normal embryonic fibroblast cultures were incubated in a medium containing 10^{-4} and 10^{-5} M ascorbic acid or control medium for 18 hours. The cells were then washed and exposed to 25 μg/ml of polyinosinic acid · polycytidylic acid for one hour. The cultures were again washed and incubated in ascorbic acid medium for ten hr. The medium was then assayed for cytopathic effects in L cell monolayers using vesicular stomatitis virus as the challenge virus, and was compared to standard reference interferon preparations. Interferon production in both types of cell cultures was significantly increased by 10^{-5} and 10^{-4} M ascorbic acid.

0921 CHEMICAL AND IMMUNOLOGICAL DIFFERENCES BETWEEN NORMAL AND TUMORAL COLONIC MUCOPROTEIN ANTIGEN. (Eng.) Gold, D. (Health Sci. Cent., State Univ. New York, Stony Brook); Miller, F. *Nature* 255 (5503):85-87; 1975.

Colonic mucoprotein antigens (CMA) were isolated from normal tissue, two primary adenocarcinomas and one liver metastasis in order to chemically and immunologically characterize the CMA. The tumor mucoproteins have reactions of partial identity with respect to each other and normal CMA using a double diffusion technique and rabbit antisera. Antisera to each tumor CMA reacted with the other two tumor CMA but not with normal CMA. None of the purified CMA reacted with an blood typing antisera. Amino acid analysis of normal and tumor CMA were similar except that all tumor CMA had less threonine, proline, valine and isoleucine. Carbohydrate and neuraminic acid analysis of the purified CMA showed great variability between the samples The results indicate that chemical and immunological differences exist between CMA obtained from normal colon tissue and colonic tumor tissues. The authors suggest that these differences may be useful in detecting colonic carcinoma.

0922 INTERSPECIES BRAIN ANTIGEN DETECTED BY NATURALLY OCCURRING MOUSE ANTI-BRAIN AUTOANTIBODY. (Eng.) Martin, S. E. (Natl. Heart Lung Inst., Bethesda, Md.); Martin, W. J. *Proc. Natl. Acad. Sci. USA* 72(3):1036-1040; 1975.

The cell surface antigen of a neural tumor cell line recognized by naturally occurring antibodies (NOA) in mouse sera is documented as a tissue differentia-

tion antigen present in brain tissue of a wide variety of species. In all experiments, serial dilutions of normal mouse serum were tested for complement dependent cytotoxicity against NB1 and 85-72 cell lines. Undiluted sera from adult A/He, AL, AKR, BALB/c, C3H/He, C3Hf/He, C57BL/6, DBA/2 and NIH mice achieved between 52.2% and 93.5% specific lysis against the NB1 cell line. Sera of C3Hf/He as well as several other mouse strains were cytotoxic for 85-72 cells. Sera of C3Hf/He mice were absorbed with homogenates of various syngeneic tissues prior to testing for cytotoxic activity against NB1 and 85-72 cell lines. The normal tissues tested did not consistently reduce cytotoxic NOA activity against 85-72 target cells. Absorption with brain or kidney tissue either markedly reduced or completely removed cytotoxic activity against NB1 tumor cells. Brain and kidney, but not liver, tissue homogenates from A/He, BALB/c and C57BL/6 mice removed anti-NB1 NOA activity from normal C3Hf/He sera. Homogenates of brain tissue of rat, guinea pig, chicken, and man and homogenates of human kidney tissue removed significant amounts of anti-NB1 NOA from normal mouse sera. NB1 cells completely removed the cytotoxic activity against NB1 cells present in the C3Hf/He serum. In contrast, the anti-NB1 NOA activity in normal C3Hf/He serum was not detectably reduced by the other cell lines, even though NOA cytotoxicity for each of these cell lines was present in normal C3Hf/He serum. The results demonstrate that the NB1 cell surface antigen recognized by NOA in mouse sera is a normal tissue differentiation antigen present in brain and kidney, but not liver, lung, muscle, spleen, or thymus mouse tissues.

0923 A MEMBRANE PERMEABILITY TEST FOR THE DETECTION OF CELL SURFACE ANTIGENS. (Eng.) Kurth, R. (Imperial Cancer Res. Fund Lab., P. O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England); Medley, G. *Immunology* 29(5):803-811; 1975.

A microtest is described which utilizes antibody-mediated release of [^{14}C]nicotinamide ([^{14}C]Na) from target cells for the detection of cell surface antigens. D4 mouse fibroblast cells derived from a sarcoma induced by the avian virus strain SRV-H in an inbred STU mouse were used in the membrane permeability test. Twenty µCi of [^{14}C]NA or 50 µCi of sodium ^{51}Cr was added to trypsin-harvested D4 cells (3×10^5 cells/ml). The cells, dispensed in 10 µl portions in sixty microwells of Falcon Microplates I, were cultured for 1-5 days, washed, and then 5 µl of either normal or diluted antiserum was added to each well. The antiserum was derived from rabbits immunized with EDTA-harvested mouse D4 cells. After 15 min, 5 µl of complement (derived from guinea pig serum) and diluted 1:4 in phosphate buffered saline (pH 7.2) was added. The incubation was terminated at varying intervals to determine the kinetics of isotope release. The optimal sodium ^{51}Cr concentration in the original cell suspension was 50 µCi of ^{51}Cr/3×10^5 cells/ml; 100 µCi ^{51}Cr gave similar results while 200 µCi ^{51}Cr was toxic for the target cells. A high specific release was obtained with 50 µCi of [^{14}C]NA/3×10^5 cells/ml, but 20 or 10 µCi of [^{14}C]NA/3×10^5 cells/ml was also found to be suitable for labeling. An increase in the background release was noted with 100 µCi of [^{14}C]NA. A final incubation time of ten minutes was sufficient for a specific release of up to 100% with [^{14}C]NA, whereas 40 min was necessary for the ^{51}Cr label. The results suggest that most, if not all, the intracellular [^{14}C]NA remained unbound and was free to diffuse; with the ^{51}Cr label, two pools were present, one consisting of freely diffusible ^{51}Cr and one fraction tightly bound to proteins. The authors conclude that the [^{14}C]NA microtest is more sensitive and faster than the ^{51}Cr release assay because most of the [^{14}C]NA is rapidly released from the target cells in the initial phase of membrane permeability changes induced by the activated complement.

0924 PROGNOSTICALLY FAVORABLE IMMUNOGENS OF HUMAN BREAST CANCER TISSUE: ANTIGENIC SIMILARITY TO MURINE MAMMARY TUMOR VIRUS. (Eng.) Black, M. M. (New York Med. Coll., N.Y.); Zachrau, R. E.; Shore, B.; Moore, D. H.; Leis, H. P., Jr. *Cancer* 35 (1):121-128; 1975.

Independently derived data regarding lymphoreticuloendothelial (L-RE) responses, skin window tests, and WBC migration were used to examine the relationship between *in vivo* immunogenicity and *in vitro* antigenicity. A WBC migration procedure had been used to test cellular hypersensitivity of breast cancer patients' WBC to autologous and homologous breast cancer tissues and to murine milk containing murine mammary tumor virus (MuMTV). The response to autologous breast cancer tissues varied with the cancer tissue and the stage of the disease at mastectomy. Immunogens appeared in the *in situ* phase of the disease, provoked prognostically favorable L-RE responses, and possessed antigenic similarity to some components of MuMTV. The data suggest that the progression of human breast cancer is accompanied or preceded by a loss of tissue immunogenicity and/or diminished specific cellular hypersensitivity.

0925 FELINE ONCORNAVIRUS-ASSOCIATED CELL MEMBRANE ANTIGEN. IV. ANTIBODY TITERS IN CATS WITH NATURALLY OCCURRING LEUKEMIA, LYMPHOMA, AND OTHER DISEASES. (Eng.) Essex, M. (Harvard Univ. Sch. Public Health, 665 Huntington Ave., Boston, Mass. 02115); Cotter, S. M.; Hardy, W. D., Jr.; Hess, P.; Jarrett, W.; Jarrett, O.; Mackey, L.; Laird, H.; Perryman, L.; Olsen, R. G.; Yohn, D. S. *J. Natl. Cancer Inst.* 55(2):463-467; 1975.

A comparative study was made of feline oncornavirus-associated cell membrane antigen (FOCMA) expression in cats with naturally occurring leukemia, cats with other diseases, healthy cats exposed to feline leukemia virus (FeLV), and cats with FeLV-induced tumors. Comparisons were also made of relative frequencies of various forms of lymphoproliferative neoplasms in cats from different geographic locations. Blood was collected from the cats and serological tests were carried out for FOCMA antibody and FeLV gs antigen (gsa). Electron microscopic examination for FeLV was performed for certain animals. It was found that more than half of 145 cats with leukemia or lymphoma were negative for FOCMA

antibody, that more than 90% had titers of 4 or lower, and that none had titers higher than 16. The low titers thus seen in cats with lymphoma and leukemia were consistent with findings in which cats given injections of feline RNA tumor viruses produced high titers when they resisted progressive tumor development but low titers when progressive tumors developed. These findings suggested that immune response to FOCMA represents immune surveillance against tumor development operating in an outbred mammalian species under natural conditions. No significant differences were found in titers for various forms of leukemia-lymphoma or for FeLV-positive as compared to FeLV-negative cats. The percentage of cats with nonregenerative anemias which were FeLV gsa positive (70%) was similar to the percentage of cats with leukemia-lymphoma from the same population that were positive. Further, 55-62% of cats with other infectious diseases, such as peritonitis and septicemia, were gsa positive. It was postulated that this was due to a predisposition to infectious diseases by the immunosuppressive action of FeLV. About 52% of Boston cats with lymphoreticular neoplasms had lymphatic leukemia, contrasting sharply with a frequency of only 3% in Glasgow cats. In the Glasgow series, the alimentary form of lymphoma was most common and accounted for about half of both lymphoma and all lymphoreticular neoplasms, whereas in the Boston series, the alimentary form constituted less than 4% of all lymphoreticular neoplasms and less than 8% of lymphomas.

0926 *IN VITRO* ASSAY OF IMMUNITY TO HUMAN WART ANTIGEN. (Eng.) Morison, W. L. (Massachusetts General Hosp., Boston, Mass. 02114). *Br. J. Dermatol.* 93(5):545-552; 1975.

The mechanisms involved in the resolution of wart virus infection and the cell-mediated and antibody responses to human wart antigen (HWA) were studied in 118 (62 female, 56 male) patients with warts. Treatment was prescribed at first attendance and included: paint applied daily, cryotherapy, or excision. Controls included 41 healthy subjects (22 females, 19 males), of which only 28 had cell-mediated immune studies performed. The *in vitro* response to HWA was assayed by the leukocyte migration inhibition test (LMI); antibody detection was accomplished by counter-current immunoelectrophoresis (CIE) and hemagglutination inhibition (HI). Most patients showed a positive cell-mediated response at or about the time of resolution of their warts, but the detectable immunity was short-lived. Of the 57 patients examined at least once after resolution of their warts, nine patients showed a positive response to wart antigen, eight of whom were clear of their warts within two months. In the remaining patients, there was no correlation between duration of treatment and degree of responsiveness to wart antigen. The majority of patients with warts had antibodies and the percentage rose both with resolution of the warts and with increase in the duration of the infection. A larger percentage of the patients were positive with the HI system than with the CIE test. No correlation between the presence of antibodies and the development of cell-mediated immunity was found. The results reinforce the finding that cell-mediated immunity, as measured by LMI, to HWA increases in some patients with the resolution of their warts. Excision and cautery did not appear to alter immunity; however, with cryotherapy, inflammatory reactions developed sufficiently to allow lymphocytes to become sensitized to HWA. Patients with paint-treatment showed an increased positive response to HWA. The author concludes that the correlation between treatment and development of cell-mediated immunity did not indicate a relation between treatment and the presence or type of antibody.

0927 ISOLATION OF MAMMALIAN TYPE C RNA VIRUS CROSS-REACTIVE ANTIGEN AND ANTIBODY BY IMMUNO-AFFINITY CHROMATOGRAPHY. (Eng.) Oroszlan, S. (Flow Lab., Inc., Rockville, Md.); Bova, D.; Gilden, R. V. *Immunochemistry* 12(1):61-66; 1975.

Rauscher murine leukemia virus (R-MuLV), feline leukemia virus (FeLV), and hamster type C virus (HaLV) were purified and guinea pig antisera were prepared to demonstrate an immunoadsorbent technique for isolation of group specific antigens (p30). CNBr (50 mg/ml) was added to Sepharose 4B with pH maintained at 11-11.5 by NaOH. Sucrose density gradient-purified HaLV was disrupted and clarified by high speed centrifugation. The soluble portion was sedimented at 40,000 revolutions/min for 18 hr. This was dialyzed and the CNBr-Sepharose was added; and slowly stirred for 18 hr at 4 C. The CNBr-Sepharose was allowed to settle; 90-95% of the p30 was bound. This was resuspended and packed in a 0.5 cm diameter column. Two ml of anti-FeLV serum was passed through the column at a rate of 0.5 ml/hr and the eluate was monitored. Elution by 1 M propionic acid resulted in protein collection (peak 2) in a tube containing 0.5 g $NaHCO_3$. When guinea pig sera showing only species-specific reactions and a heterologous goat anti-FeLV serum containing cross-reacting antibodies were reacted with disrupted virus or highly purified p30, an identity reaction was observed on gel immunodiffusion. Goat anti-FeLV showed spurring by immunodiffusion when reacted with FeLV and heterologous antigens prior to immunoadsorption on the antigen-Sepharose column, but a single band was demonstrated after immunoadsorption. It was concluded that there is a cross-reactive antigen common to type C RNA virus.

0928 SERUM α-FETOPROTEIN IN HYDATIDIFORM MOLE, CHORIOCARCINOMA, AND TWIN PREGNANCY. (Eng.) Ishiguro, T. (Kansai Med. Univ., Osaka, Japan). *Am. J. Obstet. Gynecol.* 121(4):539-541; 1975.

Serum levels of α-fetoprotein (AFP) in 130 normal pregant women, 16 women with twin pregnancies, 13 patients with hydatidiform mole, and seven with choriocarcinoma were measured by radioimmunoassay. Serum AFP was absent in 10/13 (76.9%) hydatidiform mole patients and in all seven choriocarcinoma patients. Two hydatidiform mole patients had levels of AFP below 8 ng/ml, and one patient had a high level of 105 ng/ml. During normal pregnancy, serum

AFP levels in primipara patients were almost unmeasurable in the first trimester, averaged 98.1 ng/ml in the second trimester, increased to an average of 215.8 ng/ml in the third, and decreased to 95.6 ng/ml at term. AFP levels in multipara women during the first trimester were negligible; during the second trimester, they were 62.2 ng/ml; during the third, 170 ng/ml; and at term, 95.6 ng/ml. AFP levels in twin pregnancy were significantly higher than normal in 10/14 (71.4%) women. It is concluded that abnormal AFP levels during pregnancy may indicate the presence of complications such as choriocarcinoma, twin pregnancy or hydatidiform mole, and that AFP determinations may be a valuable diagnostic aid.

0929 SERUM α-FETOPROTEIN IN PATIENTS WITH NEOPLASMS OF THE GASTROINTESTINAL TRACT. (Eng.) McIntire, K. R. (Natl. Cancer Inst., Bethesda, Md.); Waldmann, T. A.; Moertel, C. G.; Go, V. L. W. *Cancer Res.* 35(4):991-996; 1975.

Serum α-fetoprotein (AFP) levels were measured by a sensitive double-antibody radioimmunoassay in 580 patients with a variety of malignant and nonmalignant gastrointestinal diseases. Over 200 normal control subjects had AFP levels below 30 ng/ml; levels above 40 ng/ml were considered elevated. Three percent of 191 patients with colorectal carcinoma, 15% of 95 patients with gastric carcinoma, 24% of 45 patients with pancreatic carcinoma, 25% of 8 patients with biliary tract carcinoma and 70% of 73 patients with hepatocellular carcinoma had elevated serum AFP. None of 14 patients with esophageal or small bowel carcinoma had elevated levels. Only 1% of 154 patients with nonmalignant, nonhepatic gastrointestinal disease had AFP elevation. Of the 14 patients with gastric carcinoma and AFP elevation, 64% showed liver metastases or abnormal liver function (elevated Bromsulphalein or alkaline phosphatase). Three of five patients with colorectal carcinoma and 10 of 11 patients with pancreatic carcinoma and elevated AFP also showed liver metastases or abnormal liver function. Marked but temporary improvement and drop in AFP levels were observed in one patient with gastric carcinoma following 5-fluorouracil therapy. Similarly, a temporary remission and AFP drop occurred in a patient with colon carcinoma following methyl cyclohexylchloroethylnitrosourea treatment. Antigenic comparison of serum AFP in different disease conditions was made by double diffusion using a specific horse anti-AFP. It was determined that the protein responsible for elevated serum levels in stomach, pancreas, and colon was identical to AFP in serum of patients with hepatocellular carcinoma, embryonal cell carcinoma and fetal serum. AFP can serve as a serum marker to indicate the extent of certain advanced gastric or pancreatic cancers and may be potentially useful in a combination of tumor evaluating assays.

0930 EFFECT OF CHEMICAL MODIFICATION ON THE IMMUNOGENICITY OF HOMOLOGOUS α-FETOPROTEIN. (Eng.) Ruoslahti, E. (Dept. Serology and Bacteriology, Univ. Helsinki, Helsinki, Finland); Pihko, H. *Ann. NY Acad. Sci.* 259:85-94; 1975.

α-Fetoprotein (AFP) was isolated and characterized from several species and corresponding radioimmunoassays were employed to measure AFP and anti-AFP. In addition, the experimental breaking of tolerance of AFP was studied in rabbits and mice by chemical modification of the immunogenicity of homologous AFP. The AFPs of several species were found to be similar and showed immunological cross-reactions. The molecular weights as determined by gel filtration and gel electrophoresis of human, rabbit, mouse, and fetal chicken serum AFPs were 70,000, 68,000, 74,000, and 70,000, respectively, indicating that AFP is not confined to mammals. Label produced by iodination of the C-terminal CNBr peptide bound significantly by antiserum prepared against purified human AFP and to two antisera prepared against chicken AFP, indicating a certain degree of sequence homology between chicken and mammalian AFP. The existence of tolerance against AFP was confirmed by the failure of the immunization of rabbits and mice with homologous fetoprotein to elicit antibody formation, suggesting that adult lymphocytes are tolerant to autologous AFP. The AFP levels in adult humans was only 5 ng/ml, but was much higher in mice and rabbits, 100 and 170 ng/ml, respectively. The AFP of normal human serum was found to be immunologically indistinguishable from fetal AFP in humans, mice and rabbits by radioimmunoassay and immunodiffusion. Tolerance to AFP was broken by immunization with heterologous or modified AFP; antibodies cross-reactive with the rabbit AFP were produced by immunization with haptenated rabbit AFP. The autoantibody-nature of these antibodies was demonstrated by their elimination of normal human serum AFP. Rabbit AFP complexed with sheep anti-human AFP antibodies was also effective in the breaking of tolerance. These data suggest that while T-cells are tolerant to autologous AFP, some B-cells are not. The authors suggest the use of homologous anti-AFP antibodies in the immunotherapy or immunoprevention of AFP-producing tumors.

0931 ESTIMATION OF CARCINOEMBRYONIC ANTIGEN IN ULCERATIVE COLITIS WITH SPECIAL REFERENCE TO MALIGNANT CHANGE. (Eng.) Dilawari, J. B. (St. Mark's Hosp., London, England); Lennard-Jones, J. E.; Mackay, A. M.; Ritchie, J. K.; Sturzaker, H. G. *Gut* 16(4):255-260; 1975.

Carcinoembryonic antigen (CEA) levels in plasma samples obtained from 70 healthy adults, 59 patients with no evidence of colitis or malignancies, 139 patients with uncomplicated ulcerative colitis and seven patients with ulcerative colitis were determined using a double antibody technique to determine if CEA levels in colitis patients could be correlated with the presence of carcinoma or precancerous changes. Mean CEA levels in normal adults, ulcerative colitis patients and other patients did not differ. CEA levels in colitis patients did not correlate with patient age, length of disease, activity of the disease or bowel involvement. Six of seven patients with severe dysplastic changes in the rectal mucosa and six of seven patients with carcinoma complicating colitis had levels within the control hospital population range. The results indicate that plasma CEA levels are not useful in predicting cancerous changes in colitis patients.

0932 PHYSICAL CHEMISTRY AND IMMUNOCHEMISTRY OF CEA: ROLE OF THE PROTEIN PORTION OF THE MOLECULE IN DETERMINING TUMOR ANTIGENICITY. (Eng.) Banjo, C. (Montreal General Hosp., Montreal, Canada); Freedman, S. O.; Gold, P. *Ann. NY Acad. Sci.* 259:382-388; 1975.

The physical chemistry and immunochemistry of carcinoembryonic antigen (CEA) of human digestive tract adenocarcinomas and fetal digestive organs was studied. CEA was purified and its chemical composition, tumor-specific antigenic grouping, and the role of its protein portion in tumor antigenicity determined. CEA was purified by a sequence of procedures involving perchloric acid extraction, column chromatography on Sephadex 4 B and G-200, and preparative block electrophoresis on Sephadex G-25. Its molecular weight was 200,000 daltons. CEA was soluble in water, perchloric acid, and in halogenated ammonium sulfate. The carbohydrate:protein ratio of CEA was 3:1, but ranged from 5:1 to 1:1 in gastric cancer and colonic cancer tissues, respectively. The principal carbohydrate constituents of CEA was N-acetylglucosamine (25% of total wt). Variations were observed in fucose, galactose, and sialic acid contents between different preparations. Variations were also observed in the amino acid composition; however, approximately 50% of the protein is made up of asparagine, glutamic acid or glutamine, threonine, serine, and leucine. Partial hydrolysis by polystyrene sulfonic acid suggested that a heterosaccharide grouping consisting solely, or largely, of N-acetylglucosamine was important for the tumor-specific antigenic site of the CEA molecule. Additional degradation experiments suggested that either aspartic acid or asparagine and glutamic acid or glutamine may be covalently bound to the carbohydrate moiety. Cysteic acid determinations on CEA preparations and carboxymethylcysteine measurements suggested that inter- or intramolecular disulfide bridges were present and may be important for tumor antigenicity. Electrophoresis of completely reduced CEA molecules indicated that CEA is composed of a single polypeptide chain. Sequential reduction-alkylation reduced the activity of CEA by 75% to inhibit the binding of ^{125}I-labeled CEA to anti-CEA antiserum. The authors conclude that: 1) the untreated CEA molecule consists of a single protein chain that contains intermolecular disulfide bridges; 2) oxidative cleavage of these bridges results in a decreased affinity of CEA for its corresponding antibody suggesting that the tertiary structure of the protein portion of the molecule is important in CEA-anti-CEA binding; and 3) the retained capacity of the reduced and alkylated CEA to completely inhibit the standard ^{125}I-labeled CEA-anti-CEA system suggests that reduction-alkylation does not destroy the tumor antigenic determinant grouping.

0933 CARCINOEMBRYONIC ANTIGEN AND ALPHA FETOPROTEIN IN MALIGNANT TUMORS OF THE FEMALE GENITAL TRACT. (Eng.) Seppala, M. (Univ. Cent. Hosp., Helsinki, Finland); Pihko, H.; Ruoslahti, E. *Cancer* 35(5):1377-1381; 1975.

Serum samples were taken from 53 women with ovarian cancer, ten women with endometrial cancer, six women with other types of cancer, 31 women with nonmalignant gynecologic disorders, and 30 normal women to evaluate the diagnostic significance of circulating carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP) in gynecologic cancer. CEA and AFP levels were measured using double antibody radioimmunoassay procedures. With the exception of one case of benign cystic embryonal teratoma, CEA levels of greater than 5 ng/ml were associated with malignant disease. Elevated CEA levels were seen in 21% of patients with ovarian cancer and 20% with endometrial carcinoma. Elevated CEA levels were seen in ovarian cancer patients in advanced cases only. AFP levels were normal in all but one patient with adenomatous carcinoma. Ascitic fluid from one of four patients with ovarian cancer contained very high concentrations of CEA. The CEA from the ascitic fluid of this patient was indistinguishable from CEA of colon carcinoma. The results indicate that measurements of carcinofetal components are probably of little diagnostic value because increases in the levels generally occur late in the progression of female genital tract carcinomas.

0934 DETECTION OF T-CELL SURFACE ANTIGENS IN A MAREK'S DISEASE LYMPHOBLASTOID CELL LINE. (Eng.) Nazerian, K. (Agric. Res. Stn., U.S. Dep. Agric., East Lansing, Mich.); Sharma, J. M. *J. Natl. Cancer Inst.* 54(1):277-279; 1975.

An indirect membrane immunofluorescence technique was used to determine if T cells in Marek's disease (MD) lymphoma in chickens were transformed by the MD virus or had invaded the lesions as a result of an immunological reaction. Antibodies to chicken thymus cells were prepared in turkeys, and were found to stain chicken thymus cells specifically. These antibodies were applied to cultures of the MD lymphoblastoid cell line (MSB-1) derived from MD lymphoma, and to a TLT lymphoblastoid cell line established from a lymphoid leukosis tumor. All of the MSB-1 cells were stained by the antibody, 99% of chicken T cells were stained, 1-2% of chicken B (bursa) cells and none of the TLT cells. The results indicate that the MSB-1 cells transformed by MD virus were of thymus origin.

0935 T AND B ORIGIN OF HUMAN LYMPHOCYTIC LEUKAEMIA. (Eng.) Sabolovic, D. (INSERM, Res. Unit 95, Plateau de Brabois, 54500 Vandoeuvre-les-Nancy, France). *Cytobios* 12(46):79-88; 1975.

Electrophoretic mobility (EM) was used as a cell-marker for a new immunological classification of leukemias in man, and the findings were compared with those previously reported using presence of surface Ig, or capacity to form rosettes with sheep red blood cells, as cell-markers. It was found that for all of 11 cases of acute lymphocytic leukemia (ALL) in children and for 23/31 cases of chronic lymphocytic leukemia (CLL) in adults, the EM of circulating blood lymphocytes was 1.05 ± 0.005 m/sec/v cm. This EM was the same as that found for antigen-stimulated normal T cells and for cytologically normal lymphocytes in the blood of ALL patients during

early relapse. For the remaining 8/31 cases of CLL in adults, the EM of circulating lymphocytes was 0.81 ± 0.01, corresponding to that of normal circulating B lymphocytes. One exceptional case was that of ALL in an adult person for which a low EM of 0.68 was observed, together with strong immunofluorescence with an anti-Ig serum. Based on related work with the mouse, this low EM was suggested to be due to proliferation of B cell precursors. Another exceptional case was that of a high EM of 1.19 for lymphocytes from a patient with T-cell leukemia. The various findings, taken together, suggested that leukemia cells in ALL and CLL behave like highly stimulated cells. This might mean that the differences between leukemic and normal, stimulated T or B cells, are quantitative rather than qualitative.

0936 A THIRD SIGNAL IN B CELL ACTIVATION GIVEN BY TRF. (Eng.) Schimpl, A. (Institut fur Virologie und Immunbiologie der Universitat Wurzburg, Wurzburg, Federal Republic of Germany); Wecker, E. *Transplant. Rev.* 23:176-188; 1975.

Data on the biology and chemistry of the T cell replacing factor (TRF) are presented, and discussed in the general context of B cell activation. Production of TRF is studied in supernatants harvested from spleen or lymph-node cell cultures stimulated by either allo-antigens or Con A. Assuming that TRF is probably a T cell product, experiments were performed to investigate the removal of adhering cells, macrophage involvement, and the metabolic requirements of TRF production. The data are interpreted to indicate that although some TRF might be preformed and released after stimulation, the majority of the active substance depends on *de novo* protein synthesis after stimulation. An additional hypothesis suggests a linkage of the allogeneic effect factor (AEF), presumably identical to TRF, with the I region associated antigens. An autoradiography study reveals that TRF apparently acts only by directly converting already proliferating cells, although the possibility of the creation of functional T cells from undifferentiated precursors is still acknowledged. Data presented on the postulated strain specificity of T cell replacing factors remain contradictory, the discrepancies possibly due to quantitative effects. The acknowledgement of high biological activity mediated by very small quantities of TRF leads to studies of its chemistry, which thus far involve primarily purification and molecular weight determinations. Three distinguishable sequential stages of B cell activation in the course of a humoral immune response are noted and discussed, and likewise three types of probable antigens are described. It is not known whether cells not yet reactive to TRF cannot bind it or cannot recognize the signal.

0937 CHARACTERIZATION OF CYTOTOXIC EFFECTOR CELLS IN THE MOUSE MAMMARY TUMOR SYSTEM. (Eng.) Lane, M. A. (Dep. Bacteriol. Immunol., Univ. California, Berkeley); Roubinian, J.; Slomich, M.; Trefts, P.; Blair, P. B. *J. Immunol.* 114(1):24-29; 1975.

Cell types involved in the *in vitro* immune response to murine mammary tumor virus (MTV)-induced BALB/cfC3H mammary tumors were studied using techniques designed to inactivate or deplete different cytotoxic effector cells from spleen cell populations. The minimal activity of spleen cells from neonatally MTV-infected virgin BALB/cfC3H females was dependent upon the presence of T cells. Spleens from multiparous BALB/cfC3H females had a more active T cell population and, in addition, a non-T cell population which was active early in the culture period. The responses of spleen cells from multiparous BALB/cfC3H females bearing small mammary tumors were similar to those of spleen cells from tumor-free multiparous females. However, after the tumors had grown to a large size, the activity of the non-T cell population was no longer detectable. The cytotoxic activity of spleen cells from BALB/c females older than 14 weeks (supposedly not infected with MTV) was not dependent upon the presence of T cells. No activity was shown by spleen cells from BALB/c females younger than 14 weeks. Positive control spleen cells were obtained from C3H female mice who had been immunized against BALB/c histocompatibility antigens by a skin allograft; both a T cell and a non-T cell response were found. The results suggest that the non-T cell response as well as the T-cell response may be important in the host's rejection of foreign tissue. In the T cell cytotoxic response, the specificity appears to lie in the recognition by T cells of specific antigens on the surface of the tumor target cells. B cells may supply small amounts of antibody which is specific for antigen on the mammary tumor target cells, this specific antibody mediating non-T cell cytotoxicity.

0938 SPECIFIC STIMULATORY AND CYTOTOXIC EFFECTS OF LYMPHOCYTES SENSITIZED *IN VITRO* TO EITHER ALLOANTIGENS OR TUMOR ANTIGENS. (Eng.) Kall, M. A. (Univ. Washington Med. Sch., Seattle); Hellström, I. *J. Immunol.* 114(3):1083-1088; 1975.

Spleen cells (SC) or lymph node cells (LNC) obtained from normal BALB/c mice were incubated for 3, 5 or 6 days with mitomycin-C-treated sarcoma cells to study the mechanism of cell-mediated immune reactions. A/Sn mice were inoculated with Moloney sarcoma virus which induced sarcomas Y1243 and Y1175. 3-Methylcholanthrene injected i.m. in BALB/c mice induced sarcomas 1228, 1315, 1321 and 1358. As measured by a microcytotoxicity assay, LNC from BALB/c mice sensitized with A/Sn sarcoma cells killed only A/Sn sarcoma cells, while LNC from BALB/c mice immunized against BALB/c sarcoma cells did not. Lymphoid cells tested after three days of sensitization were found to increase the numbers of target cells remaining attached in six of seven experiments. The increases were specific in that they were seen only for target cells, which had been used for sensitization. No signs of cytotoxicity were seen in any experiments after three-day sensitization. Significant stimulation and cytotoxicity were observed in 45% of the cases after three and five days sensitization, resp. In 10% of the cases, stimulation was found after three days of culture without any cytotoxicity developing after six days. The results

indicate that lymphoid cells can be sensitized *in vitro* to become selectively cytotoxic to cells carrying the relevant antigens, and that antigen-specific enhancement of target cell attachment was produced by sensitization for three days *in vitro*.

0939 A COMPARISON OF HUMAN LYMPHOID CELLS IN ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC). (Eng.) Gale, R. P. (Univ. California Sch. Med., Los Angeles); Zighelboim, J.; Ossorio, R. C.; Fahey, J. L. *Clin. Immuno. Immunopathol.* 3(3):377-384; 1975.

The ability of four populations of human lymphoid cells to act as effector cells in antibody-dependent cellular cytotoxicity (ADCC) was studied. Cell populations included lymphocytes from nine normal donors, from four patients hospitalized with congestive heart failure, and from nine patients with chronic lymphocytic leukemia (CLL). The fourth group was from five established lymphoblastoid cell lines (LCL): UCLA-62, 81, 85, 91, and 101. ADCC function was correlated with the presence of a cell surface receptor for antigen-antibody complexes, i.e., an Fc receptor. Each patient and control were tested with three effector cell target cell ratios (100:1, 50:1, and 10:1). Normal and hospitalized controls exhibited comparable levels of ADCC activity and Fc receptors. CLL cells had decreased or absent ADCC activity and normal to increased percentages of cells with Fc receptors. LCL cells had no ADCC activity or Fc receptors. Thus, the presence of an Fc receptor on lymphoid cells appears to be necessary for normal ADCC activity, but is insufficient in itself to insure normal ADCC activity. Lymphocytes from CLL patients and LCL cells demonstrated a defective ability to function as effector cells in ADCC. This may be related to a defect in a subsequent step in cell-mediated cytolysis rather than to a loss of Fc receptors.

0940 EVIDENCE FOR ORIGIN OF CERTAIN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIAS AND LYMPHOMAS IN THYMUS-DERIVED LYMPHOCYTES. (Eng.) Kersey, J. (184M Jackson Hall, University of Minnesota, Minneapolis, Minn. 55455); Nesbit, M.; Hallgren, H.; Sabad, A.; Yunis, E.; Gajl-Peczalska, K. *Cancer* 36(4):1348-1352; 1975.

Lymphoblasts from 22 children (aged 6 mo - 16 yr) with acute lymphoblastic leukemia (ALL) or with malignant lymphoblastic or poorly differentiated lymphoblastic lymphoma were studied using surface markers characteristic of T and B lymphocytes. Peripheral blood lymphocytes were separated using a Ficoll-Hypaque density gradient. Lymphoid populations were preincubated with latex particles so that contaminating monocytes which phagocytized the particles could be distinguished from lymphocytes and lymphoblasts. Surface immunoglobulins were detected with fluorescein-conjugated antisera. A B-cell marker, i.e. surface immunoglobulin, was consistently absent. Fourteen of the children (64%) had lymphoblasts with one or both markers of T lymphocytes, i.e. receptors for SRBC and/or human T-lymphocyte antigen (HTLA), detectable using heterologous antithymocyte sera absorbed with B lymphocytes. Lymphocytes that carried SRBC receptors always carried HTLA receptors. However, six cases carried HTLA but not SRBC receptors. Thymus enlargement was found only in cases of ALL or lymphoma where T markers were present. Lymphoblasts carried the same markers in the same patient regardless of the site or time of collection. It is suggested that ALL may often involve T lymphocytes which differentiate prior to the development of the SRBC receptors, that the SRBC receptors may be lost from the T cells during malignant transformation, or that the sera used detected antigens in addition to HTLA.

0941 THE EFFECT OF COMMON ANTIBIOTICS ON LYMPHOCYTE TRANSFORMATION. (Eng.) Dam, W. C. (Rush-Presbyt.-St. Luke's Med. Cent., Chicago, Ill.); Malkinson, F. D.; Gewurz, H. *Experientia* 31(3):375-376; 1975.

Stimulation of ^{14}C-thymidine incorporation into lymphocytes caused by phytohemagglutinin (PHA) was measured in the presence of 4 μg/ml tetracycline, erythromycin, penicillin or with 20 mg% aspirin to determine if the antibiotics might be immunosuppressive at therapeutic concentrations. Whole blood was obtained from 26 healthy volunteers. Four-milliliter cultures of 2 x 10^5 lymphocytes/ml were treated with 250 μg PHA plus antibiotic or aspirin. After four days incubation at 37 C with 5% CO_2, lymphocyte blastogenesis was determined by adding 0.2 ml ^{14}C-thymidine (0.6 μCi) and subsequently measuring uptake by liquid scintillation. None of the antibiotics affected PHA stimulation of ^{14}C-thymidine incorporation, but 20 mg% aspirin suppressed it by 29%. The results indicate that *in vitro* concentrations of tetracycline, erythromycin and penicillin equivalent to therapeutic *in vivo* concentrations have no effect on lymphocyte response to PHA mitogen.

0942 STUDIES ON LYMPHOCYTE SENSITIZATION TO ENCEPHALITOGENIC PROTEIN IN TUMOR PATIENTS. (Eng.) Cerni, C. (Institut fur Krebsforschung d. Univ. Wien, Borschkegasse 8 a, A - 1090 WIEN/Austria); Micksche, M. *Acta Neuropathol.* [*Suppl.*] (*Berl.*) 6: 65-68; 1975.

The sensitization of lymphocytes from 18 male patients with different malignant diseases against encephalitogenic factor (EF), a basic protein isolated from human brain, was examined using the migration-inhibition test. Aliquots of lymphocyte suspensions (5 x 10^6 cells/ml) from the patients and from seven healthy male donors were incubated with different concentrations of the purified EF (25, 50, and 100 μg) for 16 hr. Seventeen of the 18 patients with malignant disease showed a specific inhibition or enhancement of the migration area of lymphocytes of more than 15%. Inhibition was observed with lymphocytes of seven patients with inoperable bronchogenic carcinomas, two with gastric carcinomas, one with melanoma, and one with lymphoma. A borderline inhibition of 14% was noted with lymphocytes of another

patient with bronchogenic cancer. Specific stimulation occurred with lymphocytes from two patients with inoperable bronchogenic carcinoma, two with intestinal carcinoma, and two with lymphoma. It is not clear from these results whether there are two different mediators of cell-mediated immunity (one that lowers the electrophoretic mobility of macrophages and one that inhibits lymphocyte migration) or whether sensitized lymphocytes produce just one mediator expressing different biological activities depending on the indicator cell and the test system. While the migration-inhibition test is sensitive and specific in demonstrating the sensitization of lymphocytes to EF, it is not a convenient diagnostic cancer test.

0943 STUDY OF THE CELLS PROLIFERATING IN PARENT VERSUS F_1 HYBRID MIXED LYMPHOCYTE CULTURE. (Eng.) Piguet, P.-F. (Univ. Geneva Fac. Med., Switzerland); Dewey, H. K.; Vassalli, P. *J. Exp. Med.* 141(4):775-787; 1975.

Mixed lymphocyte cultures (MLC) between cells of parental and F_1 hybrid origin were performed with cells from different lymphoid organs, cells depleted in T lymphocytes, and irradiated cells. The F_1 hybrids used were obtained by mating C57BL/6 and BALB/c female mice with CBA/H-T6T6 male mice. In MLC of spleen cells, karyotypic analysis revealed a strong F_1 hybrid cell proliferation (40% of cells in mitosis were of F_1 origin) regardless of strain combination. A maximum of 21% of the mitotic cells were F_1 in lymph node MLC. In MLC between cortisone-resistant (CR) thymocytes, cellular proliferation was mainly of parental origin but the number of F_1 proliferating cells increased to 5% when cultured in 5% fetal calf serum. Using parental or F_1 spleen cells depleted in T lymphocytes by use of mouse T-lymphocyte antigen (MTLA) or by treatment with anti-θ antisera plus complement, it was found that F_1 cell proliferation is entirely dependent on the presence of parental T cells. Proliferation does not require the presence of T lymphocytes among the F_1 cells. Immunofluorescence analysis of irradiated spleen blasts observed in one-way MLC showed that about 70% of the parental blasts were T blasts and 25% B blasts (containing a large proportion of plasmablasts). The same percentage of B blasts and plasmablasts occurred among the F_1 blasts but many of the T blasts bore only small amounts of T-cell antigen. About 20% of the F_1 blasts could not be calssified as T or B cells and these are possibly blasts containg MTLA in amounts undetectable by immunofluorescence. F_1 responding T cells may belong to a subpopulation performing a suppressive function because MLC lacking F_1 T cells showed increased (^3H) thymidine incorporation. The proliferation and differentiation of parental and F_1 B cells may result from unspecific, "polyclonal" triggering.

0944 AUTOGENOUS LEUKOCYTE MIGRATION IN HUMAN MALIGNANCIES. (Eng.) Elias, E. G. (Univ. Maryland Hosp., 22 S. Greene St., Baltimore, Md. 21201); Elias, L. L. *Cancer* 36(4):1393-1398; 1975.

The capillary tube technique of the leukocyte migration test was performed utilizing tumor cells and autogenous peripheral WBC from 35 patients with untreated malignancies. Migration of the autogenous WBC was inhibited in 18 of 20 patients with resectable tumors (mainly colorectal carcinomas), and no evidence of hematogenous metastasis or widespread disease (regardless of lymph node involvement) was observed. However, autogenous WBC migration was not inhibited in 14 of 15 patients with hematogenous metastasis or widespread disease, (colorectal carcinoma, melanoma, carcinoma of the lung and stomach, and hypernephroma) whether the cells were from the primary or metastatic sites. Autologous tumor extracts, and frozen-thawed cells gave almost identical results. This suggests that localized tumors are recognized as antigenic by the presensitized autogenous WBC, resulting in the inhibition. Normal tissues did not inhibit migration, presumably because they had not been recognized as antigenic.

0945 POLYMORPHONUCLEAR LEUKOCYTES IN ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY. (Eng.) Gale, R. P. (Sch. Med., Univ. California, Los Angeles); Zighelboim, J. *J. Immunol.* 114(3):1047-1051; 1975.

Human polymorphonuclear leukocytes (PMN) obtained from healthy adult donors were tested for antibody-dependent cellular cytotoxicity (ADCC) using a radiochromium release assay to determine if PMN might be involved in anti-tumor immunity. The antibody-coated target cells used were EL-4 cells and immune rabbit sera (IRABS) against them and (C58NT)D cells and immune rat sera (IRATS) against them. PMN lysis was greater than lymphocyte lysis at effector cell: target cell ratios of 50:1 and 100:1; at ratios of 10:1 and 1:1 they were the same. PMN lysis was significantly enhanced by 250 μg/ml carrageenan which completely inhibited complement-dependent cytotoxicity in the same system. Cyclohexamide, puromycin and chloramphenicol had no inhibitory effect on cytolysis; mitomycin-C, x-irradiation and heat-killed PMN also had no effect. Heat-aggregated gamma-globulin and soluble gamma-globulin were inhibitory at low and high concentrations resp. The PMN-mediated cytolysis was immunologically specific in that, in the presence of IRABS, only EL-4 cells were lysed, and in the presence of IRATS, only (C58NT)D cells were lysed. Preincubation of PMN with antisera was ineffective in inducing lysis. The results indicate that PMN lysis is mediated *via* an F_c (a crystallizable portion of the immunoglobulin molecule prepared by trypsin treatment) receptor which is inhibited by aggregated gamma-globulin at low concentrations.

0946 MIGRATION INHIBITORY ACTIVITY IN SERUM AND CELL SUPERNATANTS IN PATIENTS WITH SEZARY SYNDROME. (Eng.) Yoshida, T. (Univ. Connecticut Health Cent., Farmington); Edelson, R.; Cohen, S.; Green, I. *J. Immunol.* 114(3):915-918; 1975.

Peripheral lymphocytes obtained from six patients with extensive skin disease and 47 normal individuals were studied in order to determine if they produce macrophage migration inhibitory factors

(MIF) *in vivo* and *in vitro*. Direct and indirect macrophage migration inhibition was measured using guinea pig macrophages. MIF activity was found in sera of all six patients with Sezary syndrome, in serum from one of 10 patients with extensive dermatologic disease, and in none of the sera from normal individuals. The supernatant of lymphocyte cell cultures from three of five patients with Sezary syndrome had MIF-like activity. Gel filtration revealed that the MIF-like activity was contained in the fraction with a molecular weight of about 70,000. Supernatant of lymphocyte cell cultures from two patients with Sezary syndrome produced erythema and induration when injected into normal guinea pig skin. No reactions were seen following injections of supernatant from control cultures of normal lymphocytes. The authors suggest that the production of chemical mediators by lymphocytes (T cells) of patients with Sezary syndrome may be responsible for the extensive erythroderma seen in these patients because the lymphocytes are known to localize in the skin of Sezary patients.

0947 IMMUNOLOGIC EVALUATION OF HUMAN RENAL CELL CARCINOMA: *IN VITRO* STUDIES. (Eng.) Elhilali, M. M. (Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada); Nayak, S. K. *Invest. Urol.* 13(1):60-66; 1975.

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0996 A CASE OF LYMPHOMA WITH T-CELL CHARACTERISTICS. (Eng.) Burchardt, K. (Sydney Hosp., Sydney, N.S.W. 2000, Australia); Kelly, G. E.; Slezak, P.; Vincent*, P. C.; Gunz, F. W. *Aust. N.Z. J. Med.* 5(2):158-161; 1975.

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0999 *IN VITRO* DESTRUCTION OF TUMOR CELLS BY *CORYNEBACTERIUM GRANULOSUM* (CG) ACTIVATED MACROPHAGES [abstract]. (Eng.) Basic, I. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.); Milas, L.; Grdina, D. J.; Withers, H. R. *Fed. Proc.* 34(3):990; 1975.

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* (Chem): 664, 716, 717
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1000 CHARACTERIZATION OF HUMAN MALIGNANT MELANOMA CELL LINES. I. MORPHOLOGY AND GROWTH CHARACTERISTICS IN CULTURE. (Eng.) Liao, S. K. (Dep. Pediatr., McMaster Univ., Hamilton, Canada); Dent, P. B.; McCulloch, P. B. *J. Natl. Cancer Inst.* 54(5):1037-1044; 1975.

Seven malignant human melanoma cell lines were maintained *in vitro* for various periods of time, and their morphology and growth patterns in culture and their cytogenetic profiles were examined. One line, a metastatic amelanotic melanoma, established from a metastatic solid tumor by repeated treatment of the primary outgrowth with 0.02% EDTA solution, allowed a continuous culture of melanoma cells free of fibroblasts. All the cells differed in diameter from 20-50 μ. They were of three morphologic types: elongated dendritic, cuboidal, and triangular dendritic. Four cell lines showed varying degrees of pigmentation, and the morphology and melanin production of all lines remained relatively constant. The growth pattern of each line was evaluated by plating efficiency and saturation density. Two lines, consisting predominantly of elongated dendritic cells, failed to form distinct colonies; the cells grew in large patches, making plating efficiency impossible to determine. Both of these lines reached their growth plateau at day 4 or 5 and had a low saturation density (7-8 cells/cm^2). The saturation density of the other lines ranged from 12.4 x 10^4 to 18.5 x 10^4 cells/cm^2. Cytogenetic characterization using a fluorescent banding technique revealed only human chromosomes with gross aneuploidy. The modal chromosome number ranged from 46-78, except in one line where the range was from 50-152. Each line had 3-10 different markers, but one or two distinctive markers were present in more than 88% of the cells kers were present in more than 88% of the cells examined. Except for two lines which had originated from a single source, each specific marker or markers was distinct enough to differentiate one line from another. Karyotypes prepared from the cells of each line showed deviations of chromosomes with respect to individual number. Six lines showed an excess of chromosome 7 and six showed an excess of chromosome 22.

1001 SPREADING OF NORMAL AND TRANSFORMED FIBROBLASTS IN DENSE CULTURES. (Eng.) Cherny, A. P. (Inst. Exp. Clin. Oncol., Acad. Med. Sci. USSR, Moscow); Vasiliev, J. M.; Gelfand, I. M. *Exp. Cell Res.* 90(2):317-327; 1975.

The spreading of cells in cultures of normal embryo fibroblasts obtained from C3H mice and of M22 and L strain-transformed mouse fibroblasts was studied by electron microscopy. Normal fibroblasts formed sheets of cells up to nine layers in thickness. The cells were flattened and formed thin lamellae over other cells and over the intracellular substance. M22 cell cultures were also multilayered but the cells were not flattened, and L cells formed only monolayer cultures. M22 cell cultures were characterized by an almost complete absence of lamellar cytoplasm and L cells by a complete absence of it. The mean cell area of normal fibroblasts in dense culture was not different from that of normal fibroblasts in sparse culture, whereas in both M22 and L cultures, the cells in dense culture were much smaller than those in sparse culture. Mean cell area was also calculated in all three cell types following wounding, and cell migration into the wound area. Both transformed cell types were considerably smaller than normal fibroblasts following migration. The mean areas of all three types of unspread cells in suspension were not significantly different. It is concluded that normal fibroblasts can be induced to spread by contact of the fibroblast with other cells, the substratum (in sparse cultures) and intercellular structures, and that a deficiency in spreading of transformed fibroblasts may account for their unusual morphology *in vitro*.

1002 CLINICAL MANIFESTATIONS IN VINYL CHLORIDE POISONING. (Eng.) Suciu, I. (Clin. Prof. Dis., Cluj, Romania); Prodan, L.; Ilea, E.; Paduraru, A.; Pascu, L. *Ann. N.Y. Acad. Sci.* 246:53-69; 1975.

Clinical and biochemical investigations of 168 vinyl chloride workders from two factories are presented. Acute and subacute poisonings had occurred, replaced by chronic manifestations after the VC concentration had been decreased 22 times. Nervous manifestations found initially included euphoria (11%) giddiness (47%), and somnolence (45%); continued exposure resulted in complete narcosis (6%), headache (36%), irritability (9%), diminution of memory (13%), insomia (3%), and weight loss (28.4%). Similar results were obtained in subsequent animal experiments, and a reduction of nervous symptoms paralleled a reduction or the VC concentration. Cardiovascular manifestations included decreased values of arterial blood pressure, and simultaneous vasospastic disturbances in 47%; a continual increase in the number of cases with general and pulmonary constriction was found by the plethysmographic examination and measurement of pulmonary artery pressure. Latent disturbances were revealed, indicating the narcotic effect of large doses of VC, and clinical Raynaud's syndrome was initially found in 6%. Digestive manifestations were predominated by anorexia (23%), and subsequently included nausea without vomiting (28%), distension, pyrosis (9.4%), epigastric pain (16%), and pressure in the right hypochondrium (7%). The clinical examination revealed hepatomegaly without jaundice in 30.2%, associated with splenomegaly in 6% of the follow up cases. Tests included serum protein electrophoresis, thymol, Grienstedt, SO_4Zn, and BSP reactions; increased α-2 globulins, aldolase, and serum cholinesterase, and decreased albumins indicate that disturbances in protein metabolism may occur which precede the onset of hepatomegaly. Correlation of VC poisoning tests with the angioneurotic changes of hypothyroidism with corticoadrenal insufficiency implicate the vasomotor and metabolic centers. Pulmonary changes included a decrease in the respiratory volume and the vital capacity. Dermatological changes, reaching 80% in the initial month, mainly involved scleroderma (36%) and demonstrated the allergic sensitization mechanism on the skin and in respiratory pathways. Endocrine alterations revealed low values of the 17-ketosteroids,

thyroid hypofunction, plus sexual impotence in 24.1% of the cases. Prophylactic and therapeutic measures involving hermitization, reduction of working hours, periodic medical examinations, vitamin therapy, and reduction of toxic substances, reduced all symptoms by 2/3.

1003 BONE LESIONS AMONG POLYVINYL CHLORIDE PRODUCTION WORKERS IN JAPAN. (Eng.) Sakabe, H. (Natl. Inst. Ind. Health, Kawasaki, Japan). *Ann. N.Y. Acad. Sci.* 246:78-79; 1975.

The clinical findings from progressive testing of polyvinyl chloride (PVC) production workers are presented. Despite inconsistencies in the collecting and reporting of examination results, some tendencies were shown. Forty-nine of 1,597 workers engaged in PVC polymerization complained of troubles in the fingers; numbness was the most frequent symptom. Paresthesia, pain, and pallor also occurred. About 3% had early or developed symptoms of Raynaud-like phenomenon. X-ray examinations of 1,599 workers revealed abnormal digital phalanges in 104 cases; 80 of these, however, had a previous history of trauma. Two clear cases of occupational acroosteolysis were found. In addition, ten cases showed the tuft defect of digital phalanges, and 38 cases showed deformation of distal phalanges. Precautions included in a guide prepared in 1970 include prescribed procedures for reactor cleaning, the lowering of the threshold limit value for vinyl chloride from 500 ppm to 200 ppm, and yearly health examinations; the latter should include diagnosis of general symptoms, Raynaud's phenomenon, x-ray examination of the fingers, and liver function tests.

1004 GIANT-CELL TUMOR OF BONE: A DEMOGRAPHIC, CLINICAL, AND HISTOPATHOLOGICAL STUDY OF ALL CASES RECORDED IN THE SWEDISH CANCER REGISTRY FOR THE YEARS 1958 THROUGH 1968. (Eng.) Larsson, S. E. (Dept. Orthop. Surg., Univ. Umea, Sweden); Lorentzon, R.; Boquist, L. *J. Bone Joint Surg.* [*Am.*] 57(2):167-173; 1975.

A combined clinical and histopathological analysis of all 75 giant cell lesions recorded in the Swedish Cancer Registry from 1958 through 1968 was conducted. At reexamination, 53 cases constituted genuine giant cell tumor of bone and 20 cases were so-called giant cell variants. Seven cases were reclassified as non-osteogenic fibroma, six as aneurysmal bone cysts, two as fibroxanthomatous giant-cell tumor, and one each as giant-cell reparative granuloma, pigmented villonodular synovitis, benign chondroblastoma, myeloma, and undifferentiated sarcoma. The genuine giant-cell tumors were composed of stromal cells, giant cells, and occasional collagenous fibrils. Genuine giant cell tumors showed a significantly higher incidence in the urban than in the rural population; the recurrence rate was 42%. Patients under the age of 25 rarely had recurrences. A high recurrence rate (66%) was found among patients with tumors located in the distal end of the femur and the proximal end of the tibia. Tumors penetrating through the bone cortex were more aggressive than those located entirely within bone, regardless of tumor size and presence or absence of spontaneous fracture. A malignant course was found in 11.3% of cases, predominantly in patients with tumors in the femur. Histopathological grading was of no prognostic value. Primary *en bloc* resection with or without prosthetic replacement is recommended in patients over the age of 25. For tumors showing soft tissue involvement, either radical resection or amputation should be considered as preferred primary treatment. The same kinds of treatment should be considered also for tumors which have recurred after two previous treatments. It is concluded that histopathological grading or cytological appraisal regarding the degree of malignancy of the tumors is of no prognostic value. There is no correlation between grading and recurrence rate. It is not possible by histopathological classification to label those tumors which showed a malignant course.

1005 CEREBELLAR CAPILLARY HEMANGIOBLASTOMA: ITS HISTOGENESIS STUDIED BY ORGAN CULTURE AND ELECTRON MICROSCOPY. (Eng.) Spence, A. M. (Stanford Univ. Sch. Med., Calif.); Rubinstein, L. J. *Cancer* 35(2):326-341; 1975.

Organ culture and electron microscopy studies were conducted on a cerebellar capillary hemangioblastoma to determine the morphological evolution of the tumor. A tumor from the vermis of a 21-yr-old man with von Hippel-Lindau's disease was successfully maintained in organ culture systems on Millipore filter platforms and gelatin sponge foam matrices up to 48 days. Three cell types, endothelial cells, pericytes, and stromal cells, were identified in the original tumor on the basis of their fine structural features and their architectural relationships to vascular lumens and to the extracellular space. By light microscopy the explants showed, in the late stages, increased lipid droplets in the stromal cells and perivascular hyaline thickening. By electron microscopy, endothelial cells, pericytes, and stromal cells remained distinguishable as cell types. The stromal cells demonstrated features characteristic of the other cells such as micropinocytotic vesicles and the formation of zonula occludens junctions and hemidesmosomes. Basement membranes also became more apparent around the stromal cells. With increasing time *in vitro*, there was a striking increase in mature collagen fibers in the extracellular space. The roles of the different cell types in capillary hemangioblastoma and the histogenesis of the stromal cells are discussed in light of these observations. It is concluded that the capillary hemangioblastoma consists of multiple cell lines all of which are neoplastic and replicate in parallel with one another. Stromal cells may be regarded as an aberrant monopotential cell type which shares with the endothelial cell and pericyte a common mesenchymal, presumably angiogenic, ancestry, and may, on occasion, display morphological features, such as increased basement membrane formation and the formation of zonula occludens junctions, which recall its angiogenic lineage. Interconvertibility between endothelial cells and stromal cells does not appear to occur *in vivo* or *in vitro*.

1006 EVOLUTION OF A PRIMARY INTRASELLAR GERMINOMATOUS TERATOMA INTO A CHORIOCARCINOMA: CASE REPORT. (Eng.) Giuffrè, R. (Inst. Neurosurgery Rome Univ. Med. Sch., Italy); Di Lorenzo, N. *J. Neurosurg.* 42(5):602-604; 1975.

A case of intrasellar teratoma with a germinal structure is described. A 10-yr-old girl presented with poor visual acuity, left temporal hemianopia, pale optic discs, and a strongly positive Pandy test. Right front craniotomy revealed a tumor 1.5 cm in diameter originating in the sella and expanding above it, thus protruding between the optic nerves. Histological examination disclosed diffuse proliferations of large polygonal cells, which were relatively monomorphous with scant clear cytoplasm, and frequent tri- and binucleate cells; it was diagnosed as teratoma with germinomatous features. The patient was readmitted with emaciation and asthenia, with paralysis of the right third cranial nerve and deficits of the sixth and fifth nerves. Although the child was prepubertal and hypophysectomized, repeated radicimmuno- and bio-assays of the urinary gonadotropins revealed high values of a luteinizing hormone (LH)-like substance, which proved to be human chorionic gonadotropin (HCG). This level occasionally peaked to over 300,000 IU/24 hr. Average follicle-stimulating hormone (FSH) concentrations were 28-30 IU/24 hr, accompanied by a marked depression of all other hormonal activities. Multiple pulmonary nodules increased in size as the level of urinary HCG rose; death ensued in five months. Postmortem examination revealed an extensive recurrence of the intra- and suprasellar tumor with diffuse erosion of the base of the skull, multiple encephalic metastases, and micronodular metastases in both lungs, liver, and left kidney. Histological examination revealed a pattern typical of choriocarcinoma. The authors emphasize the absence of precocious puberty despite very high HCG levels; this may have been due to the small quantity of FSH produced, or to unresponsiveness of the target organs.

1007 ULTRASTRUCTURE OF MEDULLARY, INTRADUCTAL, TUBULAR AND ADENOCYSTIC BREAST CARCINOMAS. COMPARATIVE PATTERNS OF MYOEPITHELIAL DIFFERENTIATION AND BASAL LAMINA DEPOSITION. (Eng.) Gould, V. E. (Michael Reese Med. Cent., Chicago, Ill.); Miller, J.; Jao, W. *Am. J. Pathol.* 78(3):401-416; 1975.

Samples from medullary, intraductal, tubular, and adenocystic breast carcinomas were studied ultrastructurally, with special interest in the patterns of myoepithelial differentiation and basal laminal deposition. Samples were selected from diagnostic frozen sections, and examined with the electron microscope. The seven medullary carcinomas consisted of closely packed cells with abundant organelles, "pale" cytoplasmic matrix, frequent desmosomes, and frequent luminal structures with numerous microvilli. The bulk of the tumors consisted of irregular groups of cells without clearly delineated nest or cluster-type arrangement, and abundant lymphocytes were identified. The neoplastic cells of the five intraductal carcinomas were arranged in well-defined clusters of predominantly large, "pale" cells. Desmosomes and interdigitations were conspicuous. Delineated lamina were abundant, and numerous capillaries displaying basal lamina reduplication were present. The tubular carcinoma cells were arranged in well defined ducts; the individual cells were rather uniformly cuboidal and attached by desmosomes, the cytoplasm showed abundant organelles and dense osmiophilic bodies, and the stroma consisted of large bundles of mature, closely packed collagen. The two adenocystic carcinomas showed cells arranged in large clusters. The individual cells were very irregularly shaped, with complex cytoplasmic processes, cystic spaces distinctly corresponding to pseudocysts and true lamina, stroma of mature collagen and capillaries with reduplicated basal laminae. While myoepithelial cells were abundant in intraductal and adenocystic tumors, they were uncommon in medullary carcinomas and apparently absent in tubular carcinomas. A similar finding was seen for prominent basal laminae around neoplastic epithelium. While conspicuous myoepithelial differentiation and basal lamina deposition are important in determining the differentiated character and probable behavior of some breast carcinomas, other undetermined factors may also play a role in the clinical evolution of other breast malignancies.

1008 MALIGNANT MIXED OSTEOGENIC TUMOURS OF THE BREAST: AN ULTRASTRUCTURAL STUDY OF TWO CASES. (Eng.) Llombart-Bosch, A. (Prov. Hosp. Valencia, Murcia, Spain); Peydro, A. *Virchows Arch. [Pathol. Anat.]* 366(1):1-14; 1975.

Two cases of mixed malignant mesodermic breast tumors, with osteogenic and chondrogenic structures, were studied using optical and electron microscopy. Case I involved a 40-yr-old woman, gravida II, para II, presenting a firm tumorous mass in the upper outer quadrant of the breast; a biopsy taken during radical mastectomy disclosed a 4 cm tumor. Microscopic study revealed a complex association of tissues, including undifferentiated adenocarcinomatous tissue, angiomatous mesenchymal tissue, cartilaginous tissue, and abundant osteoid tissue. Case II was a 44-yr-old woman, gravida IV, para I, with a voluminous tumorous mass of firm consistency and plurinodular nature. Microscopic study of the 12 x 10 cm tumor revealed a neoplasm of a malignant, mesenchymal nature of predominantly fibroblastic and osteoclastic giant cells. Both tumors were highly compact, possessing abundant collagen fibers. The mesenchymatous cells included tumorous and nontumorous fibroblasts situated among dense networks of collagen, chondroblasts resembling embryonic chondroblasts in both cases, very irregular polygonal or stellated osteoblasts, and giant multinucleated cells. Undifferentiated stem cells were also present in both cases; they were regarded as the point of differentiation from remaining tumorous cells within the mesenchymal group. Electron microscopy revealed chondroblasts possessing a highly developed rough endoplasmic reticulum in active synthesis with an amorphous material contributing to the buildup of the ground substance matrix of the

tumorous cartilage. Osteoid fields with scattered osteoblasts appeared throughout the tumorous stroma and were associated with calcium deposits. Bone cell precursors of Scott's type A cells or matrix-producing cells were present in the chondroblastic or osteoblastic areas of both tumors. The ultra-structural criteria supporting an epithelial nature of adenocarcinomatous fields include the presence of very close cytoplasmic processes forming nests of cells, and club-like projections and digitations along the plasma membrane of two neighboring cells. The authors contend that all the cells in both tumors have a unique and common origin. The common point was the undifferentiated cell, with no support found for an epithelial metaplasia to mesenchymal, osteoclastic, chondromatous or osteogenic patterns.

1009 NUCLEAR MAGNETIC RESONANCE STUDIES ON HUMAN BREAST DYSPLASIAS AND NEOPLASMS. (Eng.) Medina, D. (Baylor Coll. Med., Houston, Tex.); Hazlewood, C. F.; Cleveland, G. G.; Chang, D. C.; Spjut, H. J.; Moyers, R. *J. Natl. Cancer Inst.* 54(4):813-818; 1975.

The capability of nuclear magnetic resonance (NMR) spectroscopy to distinguish between normal, diseased, and neoplastic breast tissue was investigated using both spin-spin (T_2) and spin-lattice (T_1) relaxation times. Measurements were made on a pulsed NMR spectrometer at a resonance frequency of 30.3 mHz, using 60 mg of sample for each measurement. The Carr-Purcell pulse sequence was used for T_2, and T_1 measurements were calculated by the "null" method. Values for seven normal specimens of breast tissue (682 and 35.5 msec for T_1 and T_2, resp.), 21 fibrocystic specimens (655 and 37.0 msec), eight fibroadenomas (980 and 62.5 msec), and 17 adenocarcinomas (874 and 68.6 msec) indicated that the mean NMR T_1 and T_2 of human mammary adenocarcinomas could be distinguished from those of normal breast tissue and fibrocystic disease, but not from fibroadenomas. The probability of classifying a pair of values as fibrocystic or neoplastic could be accomplished with 85% and 81% of confidence, resp. Eighty-nine percent of fibrocystic specimens and adenocarcinomas were classifiable. The values indicated that T_2 may be more discriminating than T_1 in certain states, and that a T_1/T_2 ratio could be useful in distinguishing malignant tissues from nonmalignant ones. NMR spectroscopy is a potentially useful tool for the pathologist.

1010 LOBULAR CARCINOMA ARISING IN FIBROADENOMA OF THE BREAST. (Eng.) Buzanowski-Konakry, K. (Mayo Clin., Rochester, Minn.); Harrison, E. G. Jr.; Payne, W. S. *Cancer* 35(2):450-456; 1975.

Clinical records of five patients with lobular carcinoma which arose within the epithelial component of fibroadenoma of the breast are described. The five cases occurred in a series of 4000 cases of fibroadenoma reported during a 43-yr period at the Mayo Clinic. The five patients were women, average age 45 yr. The carcinoma developed primarily *in situ* and its extent within the fibroadenoma varied greatly from minute foci of lobular carcinoma *in situ* to almost total replacement of the epithelial component of the fibroadenoma. The occurrence of lobular carcinoma *in situ* was characterized by a basic pattern of cellular prominence and fullness of rounded or elongated terminal ducts. The loosened cells filling the ducts were rounded, with a small amount of cytoplams that was granular, eosinophilic, or clear with fine vacuoles. The nuclei of these cells were somewhat enlarged, being two to three times the size of benign ductal epithelial nuclei, and had slightly irregular outlines, with moderate hyperchromatism and moderately prominent nucleoli. Mitotic figures were infrequent. The residual fibroadenomas were of mixed type (intracanalicular and pericanalicular). In all cases, the parenchyma of the breast revealed foci of ductal and lobular hyperplasia. Other changes included fibrocystic disease with aprocrine metaplasia, diffuse fibrous mastitis, papillomatosis, and comedomastitis. Treatment was surgery and prognosis was excellent. Based on this study and a review of 21 additional cases cited in the literature, it is concluded that the finding of a higher incidence of lobular carcinoma among patients with breast carcinoma in general may relate in part to the fact that lobular carcinoma and fibroadenoma occur among younger patients.

1011 PROLIFERATING AND NON-PROLIFERATING COMPARTMENTS IN CERVICAL DYSPLASIA AND CARCINOMA *IN SITU*. (Eng.) Rubio, C. A. (Institute of Pathology, Karolinska sjukhuset, Stockholm, Sweden); Lagerlöf, B. *Acta Pathol. Microbiol. Scand.* [*A*] 83(1):189-191; 1975.

Autoradiographical investigations are reported of the topographical distribution of mitotic figures in cervical punch biopsies and subsequent cone specimens. A total length of 3,200 μ of epithelium with carcinoma *in situ* was examined in each of seven cases. Areas rich in dividing cells alternated with areas devoid of mitotic figures. Injection of colchicine into 83 mice revealed that benzpyrene-induced intra-epithelial atypias also have the irregular distribution pattern. The findings suggest the existence of both proliferating and nonproliferating compartments in the cervical epithelium containing intraepithelial atypias.

1012 ANGIOGENESIS OF CERVICAL NEOPLASIA. (Eng.) Stafl, A. (Wisconsin Med. Coll., Milwaukee); Mattingly, R. F. *Am. J. Obstet. Gynecol.* 121(6):845-852; 1975.

A direct association between a growing tumor mass and new vessel growth is described in cited studies. Of 30,551 women examined colposcopically, 3,318 presented abnormal colposcopic lesions, as documented by colpo-photography. Histological examination revealed 1,092 cases of dysplasia, 223 cases of *in situ* carcinoma, 56 cases of microinvasive carcinoma, and 129 cases of invasive cancer. Vascular studies revealed a flat capillary network beneath the squamous epithelium in the benign cervix and cervical neoplasia. In normal squamous metaplasia, the vas-

cular structure of the individual papillae interconnect. In the process of atypical squamous metaplasia, however, the proliferation of horizontal capillaries close to the surface was observed. The vascular pattern of cervix represents an important diagnostic criterion of colposcopy; abnormal colposcopic findings represent a remodeling of the preexisting vascular network of the columnar epithelium, while the colposcopic appearance of the vessels is also a major diagnostic factor in predicting the histologic changes in the tissues. Whereas a limitation of colposcopy concerns false-positive ratings, the prediction of the histology was found 85% accurate. An hypothesis concerning the correlation of colposcopic and histologic changes in cervical neoplasia was presented, with disparities related to the severity of the neoplastic lesion. Observations suggest that cervical intraepithelial neoplasia represents proliferative epithelial activity with biological characteristics of cancer, and assert that carcinoma *in situ* already represents cancer in a dormant stage. The biological difference between the precancerous and cancerous cells was attributed to the ability of a malignant cell to evoke a process of neovascularization prior to invasion, a mitogenic response influenced by a tumor angiogenesis factor.

1013 OBSERVATIONS ON A MODEL OF THE BIOLOGY OF CARCINOMA OF THE CERVIX: A POOR FIT BETWEEN OBSERVATIONS AND THEORY. (Eng.) Coppleson, L. W. (Dep. Emerg. Med., Univ. Chicago, Ill.); Brown, B. *Am. J. Obstet. Gynecol.* 122(1):127-136; 1975.

A mathematical model expressing the biology of invasive cervical carcinoma is presented. States which may exist in a given woman were categorized as follows: normal, dysplasia, carcinoma *in situ*, and invasive carcinoma. The unavailability of longitudinal data on the natural course of such lesions prompted the basic assumption that longitudinal changes are reflected in latitudinal data. It was acknowledged that false negative error rates in dysplasia and carcinoma *in situ* may range from 20-40%, whereas false positive errors could not be directly determined. The current theory of invasive carcinoma of the cervix, a simple set of transitions from one defined state to another, was studied using a discrete time Markov process as the mathematical representation of the theory. The process is determined by the condition that in a given time period, a given proportion of individuals moves from one category to the next, regardless of the amount of time already spent in the category. The initial homogeneous model was found totally incompatible with the observed data, invalidating the widely accepted concept that there is some age-independent, measurable time when patients have carcinoma *in situ* before developing invasive carcinoma. A continuous model allowing all transition probabilities to change at each year, including flux in both directions and minimal net flux, was again inconsistent with some fundamental observations. The nature of the age inhomogeneity reflected the fact that invasive cancer increases with age, while dysplasia and carcinoma *in situ* decrease. Thus, the total time taken by cases passing from normal to invasive cancer decreases significantly in the later years, reflecting the fact that carcinoma *in situ* at an early age is not biologically equivalent to that at a later age. Two hypotheses based on the mathematical studies were presented: (1) carcinoma *in situ* is a mixture of two different lesions: a benign condition that spontaneously regresses and occurs mainly prior to 50 yr, and a premalignant condition seen mainly after age 50 which does not regress, and (2) carcinoma *in situ* is consistently overdiagnosed; hence, the apparent regression.

1014 JUVENILE POLYPOSIS AND GASTROINTESTINAL CARCINOMA: A STUDY OF A KINDRED. (Eng.) Stemper, T. J. (Univ. Iowa Coll. Medicine, Iowa City, Iowa); Kent, T. H.; Summers, R. W. *Ann. Intern. Med.* 83(5):639-646; 1975.

A family in which at least ten members have had single or multiple juvenile polyps of the stomach and large intestine and 11 members have had gastrointestinal carcinoma is discussed. Two patients had multiple juvenile polyps of the stomach and large intestine, and at least four patients had multiple juvenile polyps of the large intestine. Three patients had only 1-4 juvenile polyps of the large intestine, and one patient had single polyps of the jejunum and rectum. An additional patient had multiple juvenile polyps of the stomach. The earliest age at which symptoms occurred was ten years. Rectal bleeding and anemia were the most common initial symptoms. The occurrence of both generalized and colonic polyposis in the same family suggests that these are different expressions of the same disorder, rather than distinct syndromes. Gastric, duodenal, and cecal carcinomas were relatively common, and there were no cases of primary renal or sigmoid cancer. Two patients had definite juvenile polyps and cancer of the colon. One patient had an adenocarcinoma of the cecum and two adenomatous polyps resected 13 yr before the discovery of juvenile and adenomatous polyps of the rectum. In three other patients with malignancy, the type of associated polyp could not be established. In the six other patients with gastrointestinal or pancreatic carcinoma, polyps were not described. The colonic polyps in this family were much more variable in shape than the typical solitary juvenile polyp and in several patients were much more glandular. One patient had a solitary lesion resembling a typical villous adenoma, and the one small intestinal polyp in this series also resembled a villous adenoma in size, shape and glandular composition but had cystic glands and inflamed stroma in some areas. The carcinomas in this family did not relate closely to the polyps in location or temporal sequence. The pattern of occurrence of juvenile polyps and carcinoma suggests inheritance by a single autosomal dominant gene with a high degree of penetrance and pleomorphic phenotypes. An alternative hypothesis is that in this family there are two separate genes, an autosomal dominant gene coded for juvenile polyps and a closely linked autosomal dominant gene for cancer.

1015 MALIGNANT GASTRIC LEIOMYOBLASTOMA. (Eng.) Uhrich, G. I. (La Crosse Clin., Ltd., Wisconsin). *Abdom. Surg.* 17(3):73-76; 1975.

In 1964 a woman with no family history of neoplastic disease reported with pain in the left upper and lower abdominal quadrants. After preliminary radiologic study, laparotomy was done with removal of a 6 x 6 x 7 cm tumor mass as well as gastroduodenostomy; diagnosis was leiomyosarcoma of the stomach with negative lymph nodes. She returned in 1974 with a free-movable upper abdominal mass. IVP was negative and radiologic examination showed extrinsic compression deformity of the right mid-transverse colon with no stenosis of the gastroduodenostomy done in 1964. Laboratory studies were normal except for slightly elevated LDH and SGOT. Laparotomy revealed a 383 g cystic tumor measuring 10.5 x 11 x 6 cm attached to the left lobe of the liver, transverse colon and pancreas, with adhesions to the gall bladder and jejunum. Histologic examination showed cells with more rounded nuclei, some paranuclear clarity and eosinophilic cytoplasm with the diagnosis of malignant leiomoblastoma. It is suggested that long-term follow-up of leiomyoblastoma can aid in determining potential malignancy since this lesion represents a metastatic focus of the first lesion.

1016 MORPHOLOGY AND MORPHOGENESIS OF EXPERIMENTAL EPITHELIAL TUMORS OF THE INTESTINE. (Eng.) Pozharisski, K. M. (Petrov Res. Inst. Oncol., Leningrad, USSR). *J. Natl. Cancer Inst.* 54(5):1115-1135; 1975.

The morphogenesis of experimental epithelial tumors of the intestine, with particular attention to neoplasms of the descending colon, was studied in a population of 556 male, noninbred albino rats. Weekly s.c. injections of 21 mg 1,2-dimethylhydrazine dihydrochloride (DMH)/kg were given, and the rats were sacrificed at various intervals. Intestinal tumors developed in 339 of 407 rats surviving five months after the initiation of treatment. Single doses of DMH (7, 21, 40, or 200 mg/kg) were administered s.c., i.v., or p.o. to another population of 800 male and female rats. Tumors developed in different parts of the small and large intestines, and multiple tumors arose in a 4-5 cm long stretch of the descending colon in 100% of the rats, independent of dose and route of administration. Nearly 90% of all tumors arising in the duodenum, transverse and descending colon, and rectum were different types of adenocarcinoma. Mucinous and signet-ring cell carcinomas were frequent (29%) in the jejunum and ascending colon, and in the cecum (52.5%). Tumor development was marked by a widening of the proliferative zone within the crypts, indicating an enterocyte proliferation disorder. The ensuing *in situ* carcinoma became superficial cancer characterized by invasion of the lamina propria of the mucosa. The continued growth of the superficial cancer led to tumor extension through the tunica muscularis mucosa into underlying layers of the intestinal wall. The malignant growths developed *de novo*, or were not preceded by adenomatous polyps and diffuse or focal hyperplasia of the mucosa. Signet-ring cell carcinoma was marked by the accumulation of large quantities of goblet cells in the bottom of crypts. The goblet cells converted to signet-ring cells, which penetrated through the basal membrane and invaded the lymphatics and perineural spaces. The author suggests that tumors arise as a result of extension of the proliferative zone toward the superficial portions of the mucosa, and that they originate from cells that have lost their ability to differentiate. Tumor development is caused by enterocytes continuing to proliferate in the superficial layer of the mucosa, where proliferative activity is normally inhibited.

1017 PRIMARY IMMUNODEFICIENCY DISEASES AND MALIGNANCY. (Eng.) Shackelford, G. D. (Wilford Hall, USAF Medical Center, Lackland AFB, Tex.); McAlister, W. H. *Am. J. Roentgenol. Radium Ther. Nucl. Med.* 123(1):144-153; 1975.

Four children under 16 yr of age with primary immunodeficiency diseases (ataxia telangiecstasia and common variable immunodeficiency) and malignancy are reported. One case involving common variable immunodeficiency and adenocarcinoma of the stomach is described in detail. A microcephalic and markedly mentally retarded boy first presented at age eight yr with Staphylococcal pyoderma secondary to varicella. Immunodeficiency was diagnosed and the patient responded to antibiotics and large doses of gamma globulin. At age 15 yr, the subject was readmitted with progressive wt loss, weakness, abdominal pain, anorexia, and inability to retain oral fluids. Barium enema examination revealed a mass involving the transverse colon and an upper gastrointestinal series suggested a malignant process involving the entire stomach. A histoplasmin skin test was positive. Serum protein electrophoresis showed 6.3 g% total protein with albumin 49%, alpha-1 globulin 11%, alpha-2 globulin 18%, beta-globulin 10%, and gamma globulin 11%. Immunoglobulin (Ig) levels were IgG, 20 mg%; IgA, 0 mg%; IgM, 1036 mg%; and IgD, 8.2 mg%. He was given 5-fluorouracil therapy and discharged but died two months later. Autopsy revealed a poorly differentiated infiltrating adenocarcinoma of the stomach with invasion of the transverse colon and retroperitoneal soft tissue and metastatic involvement of para-aortic, bronchopulmonary, and cervical lymph nodes. Epithelial neoplasms comprise one-fourth of the malignancies in IgA deficiency but lymphoreticular tumors and leukemia are also present. Another case of common variable immunodeficiency was associated with Hodgkin's disease. The malignancies in two cases of antaxia-telangiectasia were, resp., reticulum cell sarcoma and lymphocytic lymphoma. Early diagnosis of malignancy rest on a high index of suspicion by the clinician and radiologist.

1018 JUGULAR BODY TUMORS: HYPERPLASIAS OR TRUE NEOPLASMS? LIGHT AND ELECTRON MICROSCOPICAL INVESTIGATIONS. (Eng.) Stiller, D. (Inst. Pathol., Friedrich Schiller Univ., Jena, East Germany); Katen-

kamp, D.; Kluttner, K. *Virchows Arch.* [*Patol. Anat.*] 365(2):163-177; 1975.

Twelve jugular body tumors developing from the glomus jugulare and tympanicum of the middle ear of humans were examined by light mocroscopy (nine cases), electron microscopy (six cases) and enzyme histochemical investigations (three cases) to determine whether the tumor growth pattern was hyperplastic or that of a true neoplasm. Four carotid body tumors were examined for comparison. Light microscopy revealed much cytological differentiation, the formation of a characteristic histological pattern, and infiltrative growth into the neighboring connective tissue. Electron microscopy demonstrated different cell types: tumorous cells surrounded by sustentacular cells and cells of local supporting tissue. The presence of secretory granules was noted, as well as large numbers of mitochondria and well-developed Golgi apparatus in the tumorous cells. Synaptic contacts were not seen. Enzyme histochemical studies supported light microscopy findings. It was concluded that jugular body tumors are neoplastic in nature.

1019 A HISTOLOGIC CHRONOLOGY OF THE CLINICAL COURSE OF THE KERATOCARCINOMA (SO-CALLED KERATOACANTHOMA). (Eng.) Kwittken, J. (Mt. Sinai Sch. Med., City Univ. New York, N.Y.). *Mt. Sinai J. Med. N.Y.* 42(2):127-135; 1975.

The sequence of histologic changes occurring in keratoacanthomas, from the inception to the involution or progression into a deeply invasive and/or metastasizing squamous cell carcinoma, is discussed. Examination of skin biopsies of 159 solitary consecutive keratocarcinomas led to the acknowledgement of five distinct chronological stages. The incipient stage, found in 6% of the cases, involved a slightly elevated, papillomatous, hyperkeratotic erythematous or skin-colored papule or plaque, with varying degrees of hyperkeratosis, hypergranulosis, acanthosis, and premature keratinization. The early stage (43% of cases) involved the advent of invasion beyond the basement membrane zone; the most striking additional features were dermal invasion by papillomatous proliferations of keratinizing cells, pleomorphism and mitotic activity, and an inflammatory cell infiltrate. Reaching the late stage (45% of cases), the epidermis at the periphery generally formed a lip over the sides of the depression; the rim of basophilic cells was atypical, and horn pearls and horn cysts were usually present. Both the depth of invasion and the degree of cellular atypism were extremely variable. The consequent early involutionary stage (3% of cases) was characterized by dissolution of atypical cells, cessation of mitotic activity at the base of the depression, and progressive destruction of the papillomatous squamous cell proliferations. The final stage, the late involutionary stage (3% of cases), revealed a flattening atrophy, a return to normal maturation and upward movement of the wall of the depression with replacement of the previously invaded zone by scar tissue. Simultaneous increase in the rate of cell production with epidermal hyperplasia and premature keratinization of epidermal cells appears to represent the hallmarks of an incipient stage lesion.

1020 CHARACTERISTICS OF CASES OF ANGIOSARCOMA OF THE LIVER AMONG VINYL CHLORIDE WORKERS IN THE UNITED STATES. (Eng.) Heath, C. W., Jr.; (Bur. Epidemiol., Cent. Dis. Control, Atlanta, Ga.); Falk, H.; Creech, J. L., Jr. *Ann. N.Y. Acad. Sci.* 246:231-236; 1975.

Thirteen cases of liver angiosarcoma (ASL) among vinyl chloride (VC) workers in five plants are documented, and a detailed review of seven cases is presented. All cases occurred in Caucasian males of mean age 48.2 at diagnosis; the mean total duration of VC work was 18 yr, with a mean time span of 20.3 yr from the first VC exposure to diagnosis. An etiologic association between VC work and tumor was readily inferred on the basis of the extreme rarity of ASL; whereas the expected annual incidence in the general population is 0.0014 case in 100,000 cases, the frequency among VC workers represents a risk ratio of observed to expected cases of 400:1. Clinical features of the seven cases examined extensively included relatively nonspecific presenting symptoms: abdominal mass, gastrointestinal bleeding, fatigue, weakness, and weight loss. At diagnosis, there was again considerable variability, to include liver enlargement accompanied by splenomegaly, free blood in the peritoneal cavity, and tenderness without organomegaly; abnormal liver tests, including variably increased SGOT, total bilirubin, alkaline phosphatase, LDH, and BSP values, were present in all cases. Review of pathological material showed evidence of portal fibrosis and tumor in all cases. Clinical and epidemiologic features of four cases of nonmalignant liver disease found among VC workers closely resembled those of the tumor cases. Epidemiologic features suggested a dose-response relationship, with an inverse relationship of intensity of exposure with the latent period. Despite the variable manifestations of liver disease, it is suggested that exposure to vinyl chloride monomer can produce hepatic fibrosis with angiosarcoma as a late manifestation.

1021 KARYOTYPIC ABNORMALITIES IN TRANSFORMED CHRONIC GRANULOCYTIC LEUKAEMIA. (Eng.) Sharp, J. C. (Child. Hosp., Sheffield, England); Potter, A. M.; Guyer, R. J. *Br. J. Haematol.* 29(4): 587-592; 1975.

Chromosome abnormalities in three cases of chronic granulocytic leukemia (CGL) are presented. Case I, a 45-yr-old man suffering from CGL for four yr, presented with blastic transformation, a rapidly enlarging and painful spleen, falling hemoglobin, and blast cells in peripheral blood; death quickly followed despite intensive chemotherapy. Case II was a 46-yr-old woman who had had CGL for three yr. Although she was receiving bisulphan, platelets and hemoglobin were falling; this represented an early blast crisis and intensive chemotherapy was begun. Case III, a 41-yr-old man with untreated CGL (two yr), had a hematologic status of uncomplicated CGL. A myelogram suggested an extradural mass, which was subsequently excised at laparotomy. Bone marrow from case I, and blood from cases II and III, were harvested; the chromosomes were banded using trypsin. The Ph^1 chromosome in all three patients appeared to

be formed as the result of a translocation of long arm material from one 22 to the long arm of a 9. Thirty orcein-stained metaphases and 11 trypsin-banded metaphases of Case I were analyzed. The chromosome numbers ranged from 46-49, with analysis of the banded cells showing five chromosomes involved. Thirty cells of case II were analyzed; 10 were banded, and all but one showed an isochromosome 17. Of 20 cells analyzed in Case III, two Ph^1 chromosomes and an extra 8 chromosome were found in all. Case I had all three specific abnormalities of transformed CGL, *viz:* trisomy 8, an abnormal metacentric chromosome, and a second Ph^1. Case II had the former two abnormalities, whereas Case III exhibited the latter two. It appears that an important cytogenetic step in the blastic transformation of CGL is the formation of an abnormal metacentric chromosome, with trisomy 8 being a secondary or nonspecific event. A second Ph^1 apparently arises subsequently during transformation.

1022 DIFFERENCES IN MARROW AND SPLEEN CELL KARYOTYPE IN EARLY CHRONIC MYELOID LEUKAEMIA. (Eng.) Zaccaria, A. (St. Orsola Univ. Hosp., Bologna, Italy); Baccarani, M.; Barbieri, E.; Tura, S. *Eur. J. Cancer* 11(2):123-126; 1975.

The karyotypes of 146 marrow and 89 spleen myeloid cells were simultaneously analyzed in nine patients with chronic myeloid leukemia, splenectomized at the onset of the disease. All patients but one carried the Philadelphia chromosome (Ph^1). Spleen cells were more frequently hypodiploid, pseudodiploid, and hyperdiploid. When all metaphases carrying one or more extra chromosomes (pseudodiploid plus hyperdiploid) were pooled, the difference was even more striking: 22.4% in the spleen *versus* 11.5% in the marrow. Spleen cells showed a more frequent loss of C and possibly of D chromosomes, and a more frequent gain of F and possibly of G chromosomes, as compared to marrow. The cytogenetic patterns of the evolution of chronic myeloid leukemia towards blastic crisis indicate increasing aneuploidy, emergence of hyperdiploid variants and progressive gain of C, F, and G (Ph^1) chromosomes. The data are consistent in part with the hypothesis that the spleen plays a role in the development of blastic crisis, and indicate that combined marrow and spleen chromosome investigations, performed later during the course of chronic myeloid leukemia, could be useful in better defining the role of the spleen and assessing its importance.

1023 DISTINCTIVE CYTOPLASMIC INCLUSIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA. (Eng.) Cawley, J. C. (Dep. Med., Univ. Cambridge, England); Emmines, J.; Goldstone, A. H.; Hamblin, T.; Hough, D.; Smith, J. L. *Eur. J. Cancer* 11(2):91-92; 1975.

Distinctive cytoplasmic inclusions are described in two of a series of 30 cases of chronic lymphocytic leukemia. The inclusions could not be identified with any certainty in the Romanowsky preparations, and were only detectable during ultrastructural examination. On longitudinal section, the inclusions were composed of a central space flanked by two rows of approximately parallel fibrils or lamellae interspersed with ribosome-like particles. In transverse section, the inclusions had a central space surrounded by several concentric lamellae. In both longitudinal and transverse sections the inclusions were frequently partially or completely surrounded by strands of rough endoplasmic reticulum. The morphology of those lymphocytes containing inclusions in no way differed from the other lymphocytes present. In both the cases of chronic lymphocytic leukemia, the inclusions were present in less than 10% of the lymphocytes. The existence of the inclusions in two cases of a series of 30 suggests that they may be more common in chronic lymphocytic leukemia that has been previously recognized. This study confirms previous findings that the inclusions are present in both chronic lymphocytic and leukemic reticuloendotheliosis.

1024 DECREASED LYMPHOCYTE ADENOSINE DEAMINASE ACTIVITY IN ACUTE LYMPHOCYTIC LEUKEMIC CHILDREN AND THEIR PARENTS. (Eng.) Zimmer, J. (Wayne State Univ. Sch. Med., Detroit, Mich.); Khalifa, A. S.; Lightbody, J. J. *Cancer Res.* 35(1): 68-70; 1975.

The hypothesis that some individuals who develop neoplasia may have immune systems unable to respond maximally to an antigenic challenge because of altered adenosine deaminase levels was examined. Adenosine deaminase was measured in the lymphocytes of 26 children with acute lymphocytic leukemia (five boys and eight girls newly diagnosed or in relapse, eight boys and five girls in remission), 25 parents, nine normal children (four boys and five girls) and 23 normal adults. Median ages were eight yr, ten months for newly diagnosed or relapse patients, 3.5 months to three yr for remission patients, 33 years for parents, and 28 years for normal adults; the normal children were of the same age groups as the patients. No significant difference was found between the mean value (12.9 ± 5.1) for adenosine deaminase in the normal adults and the mean value (10.4 ± 2.5) in normal children. The difference between the mean value of the normal adults and the mean value (7.4 ± 3.9) of parents was significant ($p < 0.01$). The difference between the mean value of the remission patients (6.8 ± 3.7) and that of the control children was significant ($p < 0.02$). The difference between the mean value of the relapse patients (3.8 ± 2.9) and that of the normal children was highly significant ($p < 0.01$). No correlation was found between age or sex and adenosine deaminase activity. Although altered adenosine deaminase levels in relapse patients could be explained by the additional malignant blast cell population, no correlation was seen between the percentage of blasts in the peripheral blood and adenosine deaminase activity. Chemotherapy and transfusions may also influence adenosine deaminase activity in both relapse and remission patients. Because these explanations would not apply to parents, it is suggested that the altered adenosine deaminase levels are genetically determined.

1025 PERSISTENT LYMPHOCYTOSIS WITH CHROMOSOMAL EVIDENCE OF MALIGNANCY. (Eng.) Brody, J. I. (Grad. Hosp., Univ. Pennsylvania); Burningham, R. A.; Nowell, P. C.; Rowlands, D. T., Jr.; Freidburg, P.; Dainele, R. P. *Am. J. Med.* 58(4):547-552; 1975.

An inappropriate, sustained and absolute lymphocytosis in a 21 year-old man was investigated. An attempt was made to define the more detailed features of the lymphocytes and their T and B cell subpopulations. The tests used included the study of lymphocyte and complement rosette formation, immunofluorescence, *in vitro* phytohemagglutinin (PHA) stimulation, and ^{14}C-cyclophosphamide binding assays. The results indicated that the lymphocytosis was mainly, but not solely, due to an absolute elevation in circulating T lymphocytes. An aneuploid cell line with 47 chromosomes, presumably T cells, was also detected in PHA-stimulated cultures. The lymphocyte-infiltrated bone marrow, anemia, hepatosplenomegaly, and pharyngeal lymphoid tumor of the patient indicate that the lymphocytosis is more than a benign, unremitting leukemoid reaction. The clonal proliferation of lymphocytes with an abnormal karyotype strongly suggests that this is a potentially malignant condition. Due to the chromosomal aberration at this patient's age and point in disease, it is suggested that he may be a clinical prototype for others with lymphoreticular tumors which remain undetected at their onset.

1026 AMERICAN BURKITT'S LYMPHOMA: A CLINICO-PATHOLOGIC STUDY OF 30 CASES. II. PATHOLOGIC CORRELATIONS. (Eng.) Banks, P. M. (Natl. Cancer Inst., Bethesda, Md.); Arseneau, J. C.; Gralnick, H. R.; Canellos, G. P.; DeVita, V. T., Jr.; Berard, C. W. *Am. J. Med.* 58(3):322-329; 1975.

The hematologic, cytologic, and pathologic findings among 30 American patients afflicted with undifferentiated Burkitt's lymphoma are reviewed. Diagnostic biopsy sections, exfoliative cytology specimens, and bone marrow aspirates from these 30 patients, predominantly Caucasian, mean age 11.5 yr, indicated the tumor was composed of a uniform population of undifferentiated cells, with an abundance of mitotic figures. Methyl green-pyronine stain demonstrated intense cytoplasmic pyroninphilia of all neoplastic cells, with numerous clear intracytoplasmic vacuoles; these vacuoles appeared clear with Wright's stain, and indicated the presence of neutral lipid *via* oil red O stain. At 17 postmortem examinations, the tumor was usually widely disseminated, affecting two or more gastrointestinal organs, including hepatic (12 patients), renal (16), pulmonary (11), and central nervous system (9) involvement. Organ systems often spared by other malignancies were frequently involved, including cardiovascular (6), musculoskeletal (6), integumentary (4), and endocrine (6) organs, but hemolymphatic organs were generally excluded. Bone marrow involvement (12 cases) was always in the form of replacement of normal elements by cohesive expanses of tumor cells. Chemotherapeutic agents appeared to alter the histologic appearance of the tumor, varying from diffuse necrosis within 48 hr of initial therapy, to extreme pleomorphism of tumor cells after nine months; in one instance these agents caused almost complete eradication of lymphoma at the original site. Accompanying the older median age (11) and the greater male:female ratio (2:1) of the American patients studied, *versus* those of East Africa was a predominance of abdominal and pelvic tumor sites, and lower incidence of jaw tumor. The anatomical distribution of tumor at the time of initial therapy was seen as a major determining factor in predicting long-term remission; extensive abdominal and/or pelvic tumor, or tumor presence in the bone marrow or cerebrospinal fluid was invariably associated with short survival. The best prognosis came when the tumor was confined to a single site, with no evidence of either central nervous system or bone marrow involvement.

1027 MALIGNANT LYMPHOMAS OF FOLLICULAR CENTER CELL ORIGIN IN MAN. II. ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES. (Eng.) Glick, A. D. (Vanderbilt Univ. Sch. Med., Nashville, Tenn.); Leech, J. H.; Waldron, J. A.; Flexner, J. M.; Horn, R. G.; Collins, R. D. *J. Natl. Cancer Inst.* 54(1): 23-36; 1975.

Human lymphoma cells were studied to determine whether they have the ultrastructural and cytochemical characteristics of follicular center cells. Of 23 malignant lymphomas studied, 21 were follicular center cell lymphomas, one was a Burkitt's lymphoma, and one was a large-cell malignant lymphoma. Most cells in normal reactive lymph nodes or tonsils were either cleaved lymphocytes, non-cleaved lymphocytes, macrophages or dendritic cells. The cleaved lymphocytes were small (5-10 μ in diameter) with inconspicuous or absent nucleoli, and singly distributed ribosomes; or large (7-14 μ in diameter) with more prominent nucleoli and polyribosomal aggregates. The noncleaved cells (70-30 μ) had large round or oblong nuclei and were filled with polyribosomal aggregates and long segments of rough endoplasmic reticulum. Macrophages had multiple lysosomal granules, phagosomes, and numerous segments of smooth and rough endoplasmic reticulum. Dendritic cells had elongated cytoplasmic processes associated with desmosomal junctions. Large macrophages with dense cytoplasmic staining for the esterase were interspersed in a background of negatively staining lymphoid cells. In the malignant lymphomas, one type of follicular center cell usually comprised greater than 90% of those counted. Seven were composed predominantly of small cleaved cells with less heterochromatin margination and more nucleoli than their normal tissue counterparts. Nine tumors were predominantly large cleaved cells; nuclear folding and cleavage planes were more exaggerated than normal. Two tumors contained mainly small uncleaved cells, and five contained large uncleaved cells, as did their normal counterparts. Randomly interspersed macrophages were in all tumors. Cytochemical studies showed an increase in the uniformity of lymphoid cell types in the smears. These findings support the concept that malignant lymphomas derive from B cells of follicular center cell origin.

1028 NODULAR LYMPHOMA: AN ULTRASTRUCTURAL STUDY OF ITS RELATIONSHIP TO GERMINAL CENTERS AND A CORRELATION OF LIGHT AND ELECTRON MICROSCOPIC FINDINGS. (Eng.) Levine, G. D. (Stanford Univ. Med. Cent., Calif.); Dorfman, R. F. *Cancer* 35(1):148-164; 1975.

The ultrastructural findings in 16 cases of nodular lymphoma are described and compared with normal germinal centers in order to determine the possible germinal center origin of the lymphomas. Long, branching desmosome-associated dendritic reticulum cells, characteristic of germinal centers, were found in all 16 cases. Desmosomes were observed only between dendritic cells, and were seen in only one of seven cases of diffuse lymphoma. Cells comprising the nodular lymphomas share cytologic features (e.g. nuclear blebs and marked nuclear indentations) with germinal center cells. It appears that nodular lymphomas have a close anatomical relationship to germinal centers, although their actual origin from these sites is not established. They are composed of lymphoid cells; there is no evidence to indicate that nodular lymphomas are derived from dendritic cells. Light and electron microscopic findings were compared to establish the nature of large histiocyte-like cells in the nodular lymphomas. Many of these cells appear to represent transformed lymphocytes rather than histiocytes. They are more numerous in so-called mixed histiocytic-lymphocytic lymphomas. The existence of a mixed lymphocytic lymphoma within the spectrum of the nodular lymphomas is questioned. It is suggested that a cytologic continuum exists between cells interpreted as poorly differentiated lymphocytes and those identified as histiocytes.

1029 ONCOCYTOMA (MITOCHONDRIOMA) OF THE PAROTID GLAND: AN ELECTRON MICROSCOPICAL STUDY. (Eng.) Sun, C. N. (Univ. Arkansas Sch. Med., Little Rock); White, H. J.; Thompson, B. W. *Arch. Pathol.* 99(4):208-214; 1975.

An oncocytoma of the left parotid gland, removed from a 61-yr-old Caucasian man was studied with the electron microscope. The tumor cells were found arranged in cords and alveolar-like clusters; typical oncocytes, and condensed or dark oncocytes were both found in the latter. The features of the typical oncocytes included a large number of tightly packed mitochondria completely filling the entire cytoplasm, sparse organelles other than mitochondria, an increased number and length of cristae, and oval or spheroid nuclei composed of a nucleolus and interchromatic granules. The condensed oncocytes were also filled with mitochondria, which showed evidence of degeneration and fusion. The nuclei were irregular shaped and dense with many inclusions and glycogen granules. The nuclear envelope was occasionally fused with the mitochondria. Regions of connective tissue were seen between the tumor cell clusters. Similar mitochondria richness had been previously reported in other oncocytic tumors; it was thus speculated that such a tumor might represent an intracellular "neoplasm" of mitochondria proliferating at the expense of the cell's own economy.

1030 CYTODIAGNOSIS OF ACINIC CELL CARCINOMA: ULTRASTRUCTURAL STUDY OF MATERIAL OBTAINED BY FINE NEEDLE ASPIRATION BIOPSY. (Eng.) Woyke, S. (Med. Acad., Szczecin, Poland); Olszewski, W.; Domagala, W.; Marzecki, Z. *Acta Cytol. (Baltimore)* 19(2):110-116; 1975.

Cells of acinic cell carcinoma obtained by fine-needle aspiration biopsy were observed by electron microscopy; these observations were applied to the cytodiagnosis of this carcinoma. The material for cytologic examination was obtained from a subcutaneous tumor in the right temporal region of a 60-yr-old man. The patient had previously presented a tumor of the right parotid gland, and had experienced recurrence and radiation treatment. Light microscopic examination of the primary salivary gland tumor and its recurrences revealed a tissue composed of polyhedral cells with abundant, finely granular cytoplasm which were distinctly basophilic. Cytological observations of blood-stained fluid revealed elements of peripheral blood among clumps of epithelial cells with abundant, granular and basophilic cytoplasm. Low magnification electron microscopy showed both grouped and isolated cells, distinguished by the presence or absence of secretory granules; the latter contained abundant cell organelles. A transitional form of cell was also observed. Electron microscopy revealed a specific type of secretory granules in many cells. In some places the cells were arranged in irregular glandular structures. The potential of electron microscopic study in providing information pertinent to the differential diagnosis was noted.

1031 ULTRASTRUCTURE OF "PINEOCYTOMA". (Eng.) Nielsen, S. L. (Univ. California Sch. Med., San Francisco); Wilson, C. B. *J. Neuropathol. Exp. Neurol.* 34(2):148-158; 1975.

The electron microscopic features of a rapidly growing human pineocytoma are defined and compared with normal mammalian pinealocytes. Tumor masses were removed, in two separate operations, from a 32-yr-old woman; tissue from both surgical procedures was identical in appearance and are described together. Light microscopy revealed a distinctive arrangement of cells, numerous thin-walled vessels, and minimal nuclear pleomorphism and inconspicuous mitotic figures in all areas. Examination of the ultrastructure of the tumor showed nuclei separated from the vessel walls by numerous cell processes and characteristic astrocytic processes. Individual tumor cells exhibited some degree of nuclear uniformity; round-to-oval nuclear contour was often altered by deep invaginations of cytoplasm, and nucleoli were not prominant. The perikaryon was rich in organelles, including large mitochondria, aggregates of ribosomes, irregularly-directed microtubules and filaments, smooth and rough endoplasmic reticulum, and centrioles. The cell membrane disclosed frequent invaginating vesicular structures; cellular organelle contents were much less complex farther from the perikaryon. Comparable ultrastructure of a baboon pineal gland was presented, including consideration of perikaryon, microtubules, and filaments. Features noted as identifying the human

tumor included the grouping of tumor cells into rosettes with complex fibrillar centers, plus the orientation about blood vessels and their rare, club-shaped, silver-positive processes. The ultrastructural similarity of the pineocytoma cells to neurons, as well as the presence of cell processes indistinguishable from those of fibrous astrocytes, may serve as two criteria in the implication of differentiation along the two cell lines.

1032 SYNAPTIC LAMELLAE IN RETINOBLASTOMA. (Eng.) Radnot, M. (Semmelweis Univ. Med. Sch., Budapest, Hungary). *Am. J. Ophthalmol.* 79(3): 393-404; 1975.

Numerous synaptic lamellae and synaptic vesicles were observed during the electron microscopic examination of the retinoblastoma of a 21-month-old girl. The length of the lamellae varied between 0.1 and 0.5 μ. Occasionally, several lamellae formed rows adjacent to each other. In other places only single lamellae were found. In most sections examined lamellae and vesicles were found in all cells joined together; rarely were lamellae in one cell. An invagination characteristic for photoreceptor synapses was observed in one place but was not found in any other cell. The sizes of the lamellae in this case resembled those in the synapses of the inner plexiform layer of photoreceptors. Longer lamellae were observed occasionally. Neither of the cellular junctions corresponded to the synapses in the normal retina, regarded as imitations of the normal synapses. They did not correspond to normal retinal structures either in size or in the composition of the pre- and postsynaptic components. The presence of synaptic lamellae and vesicles unequivocally proves the neural origin of the tumor, because such structures are never found in glial cells.

1033 AN EXPERIMENTAL MOUSE TESTICULAR TERATOMA AS A MODEL FOR NEUROEPITHELIAL NEOPLASIA AND DIFFERENTIATION. I. LIGHT MICROSCOPIC AND TISSUE AND ORGAN CULTURE OBSERVATIONS. (Eng.) VandenBerg, S. R. (Stanford Univ. Sch. Med., Calif.); Herman, M. M.; Ludwin, S. K.; Bignami, A. *Am. J. Pathol.* 79(1):147-168; 1975.

The various stages of divergent neuroepithelial differentiation were studied in the solid transplant of a mouse teratoma, OTT-6050, produced from an intratesticular implant of a six-day 129/SV embryo into the testes of an adult F_1 (A/HE x 129). Syngeneic hosts were five to eight week-old 129/J female mice; both ascitic and solid forms were cultured, and the anti-glial fibrillary acidic (GFA) protein fluorescent antibody test was carried out. Gross findings in mice injected with ascitic tumor fluid included numerous solid implants, consistently including hepatic and splenic serosal implants, and solid tumors in the anterior abdominal s.c. space. Grossly, the tumors were lobulated, but not encapsulated, and were frequently cystic with variable areas of necrosis. Microscopic observations showed that the ascitic fluid contained cells arranged in two types of structure, embryoid bodies, and sheets of larger, compactly arranged polygonal cells. The solid tumors that developed from ascitic tumor fluid contained tissues derived from the three classic germ layers intermixed without definite organization; these contained epithelial derivatives, glandular differentiation, primitive mesonephric tissues, mesodermal tissues, and differentiation involving derivatives of two or more germ layers in an organoid relationship. The solid tumors, containing 80 to 90% neuroepithelial cells, showed four main patterns of differentiation: foci of stratified low columnar or cuboidal epithelial-like cells arranged in papillary and tubular formation; highly cellular foci of small, darkly staining cells with round or slightly ovoid nuclei; areas of enlongated uni- and bipolar primitive neuroglial cells; and various stages of ganglionic differentiation. The chief characteristic of neuroepithelial differentiation in the tumor line included its discovery in solid tumors only, as a part of a divergent differentiation from multipotential stem cells, its extensiveness, involving 80-90% of the cells, and the presence of all stages of divergent neuroepithlial differentiations. The stages of differentiation were reported to correspond to three general types of human central nervous system tumors, which suggests this as a model for neuroepithelial differentiation and study of both normal and neoplastic neurocytogenesis.

1034 ATYPICAL GERM CELLS IN THE ADJACENT "NORMAL" TISSUE OF TESTICULAR TUMOURS. (Eng.) Skakkebaek, N. E. (The Laboratory of Reproductive Biology and Fertility Clinic, University Department of Obstetrics and Gynaecology, Rigshospitalet, Copenhagen Ø, Denmark). *Acta Pathol. Microbiol. Scand.* [*A*] 83(1):127-130; 1975.

The discovery of atypical intratubular germ cells in testicular biopsies from two infertile men prompted testing of the hypothesis that the atypical cells represented a carcinoma *in situ* of the testis. Histological sections from testicular tissue of 40 patients, orchiectomized because of unilateral testicular tumors, were studied. Twenty-two of the preparations contained residual testicular tissue, 17 of which showed tubules with abnormal intratubular germ cells. The atypical cells were larger than normal germ cells, were located close to the basement membrane, and had a nucleus of 10 μ with grossly fragmented chromatin. Tubules with atypical germ cells were found in six testes with embryonal carcinoma, three with terato-carcinoma, one with combined terato-carcinoma and seminoma, and seven with seminoma. The study supported the assumption that the atypical germ cells represent a carcinoma *in situ*; 77% of the tumors examined, mainly embryonal carcinomas and seminomas, were accompanied by such atypical intratubular germ cells in the surrounding testicular tissue. The atypical germ cells may represent malignant germinal cells capable of developing into different types of germ cell tumors.

1035 THE HISTOGENETIC-EMBRYOLOGIC BASIS FOR REAPPEARANCE OF ALPHA-FETOPROTEIN IN ENDODERMAL SINUS TUMORS (YOLK SAC TUMORS) AND TERATOMAS. (Eng.) Teilum, G. (The University Institute of Pathological Anatomy, Rigshospitalet, University of Copenhagen, Denmark); Albrechtsen, R.; Norgaard-Pedersen, B. *Acta Pathol. Microbiol. Scand.* [*A*] 83(1):80-86; 1975.

The mechanism of neosynthesis of alpha-fetoprotein (AFP) in patients with testicular, ovarian, and extragonadal germ cell tumors is discussed. The cellular bases of AFP production in ontogenesis and in malignancy was considered to be of decisive importance. Sequential reports of patients with ovarian and testicular tumors diagnosed as specific endodermal sinus tumors revealed that the production of AFP closely paralleled the amount of the visible malignant tumor tissue which fluctuated in the course of the diseases. The pure forms of endodermal sinus tumor was most often found in the ovary of young women and children, and in the testis of infants. Immunofluorescent studies revealed AFP-specific fluorescence in epithelium of endodermal origin lining cysts and tubules. AFP was also contained in the intra- and extracellular PAS-positive hyaline globules found in many endodermal sinus tumors. It is concluded that the histogenetic classification of germ cell tumors (endodermal sinus tumor, choriocarcinoma, and teratoma) offers a reasonable explanation of the neosecretion of AFP in endodermal sinus tumors and teratocarcinomas. According to this classification, neither the teratoid component nor the undifferentiated embryonal carcinoma component is responsible for the AFP synthesis of the tumor. Rather, the pure, embryologically highly differentiated endodermal sinus tumors are the most active AFP-producers, owing to yolk sac elements.

1036 THE SYNDROME OF MULTIPLE MUCOSAL NEUROMAS AND MEDULLARY THYROID CARCINOMA IN CHILDHOOD. IMPORTANCE OF RECOGNITION OF THE PHENOTYPE FOR THE EARLY DETECTION OF MALIGNANCY. (Eng.) Brown, R. S. (Montreal Child. Hosp., Canada); Colle, E.; Tashjian, A. H., Jr. *J. Pediatr.* 86(1):77-83; 1975.

Three new case studies of unrelated female adolescents with multiple mucosal neuroma are reported. The possible significance of serum calcitonin concentrations and genealogic data in the recognition of medullary thyroid carcinoma is presented. Serum calcitonin of blood drawn simultaneously from venous drainage of the tumor and a peripheral vein was measured by radioimmunoassay. Postoperative samples were serially obtained, including provocative testing with four hr i.v. infusion of calcium (15 mg/kg). In two cases, initial calcitonin concentrations of 1.2 and 0.82 ng/ml were well above values measured in normal subjects; venous blood draining the tumor contained over 100 times the concentration of the peripheral venous sample. Two patients had bilateral tumors; the other had a single tumor. Eighty percent of the tumors were found in the upper lobe. The thyroid tumors of all three patients were histologically compatible with medullary carcinoma of the thyroid. Of eight family members examined, neither phenotypic manifestations of the syndrome nor abnormal serum calcitonin levels were exhibited. The author suggests the three cases represent offspring of spontaneous mutations whose children bore a 50% chance of inheriting the trait. The distinctive facies are recognizable in very early childhood; physicians should be aware of this "phenotype of malignancy." Measurement of serum calcitonin level was said to offer a unique means of early detection of this thyroid malignancy. The author notes the possibility that other neuroectodermal tissues might be responsible for this production and secretion of calcitonin.

1037 EXPERIMENTAL TUMOR INDUCTION IN A CIRCUMSCRIBED REGION OF THE HAMSTER TRACHEA: CORRELATION OF HISTOLOGY AND EXFOLIATIVE CYTOLOGY. (Eng.) Schreiber, H. (Dep. Pathol., Univ. Chicago, Ill.); Schrieber, K.; Martin, D. H. *J. Natl. Cancer Inst.* 54(1):187-197; 1975.

Through a specially designed catheter, a 1% solution of *N*-nitroso-*N*-methylurea (NMU) was applied to a 6 mm length of trachea in 48 male Syrian hamsters. The cytologic and histologic progression of the induced carcinogenesis is described. NMU was dissolved in 10% ethanol-water and administered to the methoxyflurane anesthetized animals with 30 min. The catheter was introduced through the mouth into the larynx, and 1 ml of solution was delivered to the length of trachea over 5 sec. Eighteen controls were treated in the same manner with 10% ethanol-water. Cytologic samples were obtained. Interrupted serial sections of the trachea and larynx were made at 50 μ intervals. Two deaths occurred after the first and third treatments; the animals demonstrated severe tracheitis with hyperplastic and dysplastic epithelial changes. Nine treatments resulted in epithelial dysplasia consisting of undifferentiated cells with foamy or basophilic cytoplasm. Many undifferentiated, irregular cells of varying size were seen; nuclear cytoplasmic ratio was usually normal. After ten weeks, sacrificed animals demonstrated many squamous metaplastic cells, an increased nuclear cytoplasmic ratio and changes which were classified cytologically as moderate to marked atypia. There was a loss of polarity and areas of keratinization along the treated area. At the end of the exposure period, all hamsters had developed tracheal carcinomas, with 75% dying between the 15th and 20th weeks of the experiment. Fifteen had epidermoid, and three had large cell anaplastic carcinomas. Malignant cells had a significantly increased nuclear cytoplasmic ratio, marked pleomorphism and a coarse chromatin pattern. Controls demonstrated hyperplasia, but no atypia or tumors. This method is a promising tool for studies of respiratory tract carcinogenesis.

1038 RELATION OF LICHEN SCLEROSUS ET ATROPHICUS OF THE VULVA TO DEVELOPMENT OF CARCINOMA.

(Eng.) Hart, W. R. (Armed Forces Inst. Pathol., Washington, D. C.); Norris, H. J.; Helwig, E. B. *Obstet. Gynecol.* 45(4):369-377; 1975.

The malignant potential of *lichen sclerosus et atrophicus* (LS&A) of the vulva and its relation to carcinoma were evaluated through the study of 107 cases of LS&A of the vulva and peritoneum. Follow up studies on 92 cases were used as a basis of prognosis and treatment. Microscopic slides of 107 examples of LS&A were evaluated for the presence of squamous hyperplasia and dysplasia; clinical findings were abstracted from the patients' charts. All patients were Caucasians of median age 47 yr; 53 were over 50 yr. The available menstrual history revealed 7% premenarchal, 7% menopausal, and 61% postmenopausal at the time of diagnosis; a history of abnormal uterine bleeding was recorded in 22%. The most common symptom was pruritis (68%), in addition to a burning sensation, vaginal discharge, bleeding from skin lesions, and/or genitourinary tract symptoms. Multiple lesions were found in 87% of the patients, 81% involving both sides of the vulva; specific anatomic sites, in order of decreasing frequency, were the labium minus, labium majus, clitoris, perineum or perianal region, posterior fourchette, vestibule, and mons pubis. At the time of diagnosis, 5% had a synchronous invasive carcinoma of the vulva or peritoneum. The most prominent and consistent pathological finding was a broad zone of peculiar homogenization and/or edema of the papillary and reticular dermis; hyperkeratosis and keratotic follicular plugging were common. Areas of squamous hyperplasia adjacent to atrophic epidermis coexisted with the LS&A in 35% of the patients. A follow up study,with a median length of 9.4 yr, of 92 patients revealed that only 1 (1%) of the patients with biopsy-proven LS&A subsequently developed a carcinoma of the vulva. These results indicate that LS&A of the vulva and perineum has little or no malignant potential. The LS&A apparently had no direct responsibility for the development of the dysplasia or carcinoma found in 5% of the 107 studied, because no overlaid areas occurred. In view of the low incidence of vulvar carcinoma developing in patients with LS&A of the external genitalia, and the high recurrence of LS&A following vulvectomy, a conservative treatment after definitive diagnosis is suggested.

1039 BILATERAL NEONATAL WILMS' TUMOR WITH B-C CHROMOSOMAL TRANSLOCATION. (Eng.) Giangiacomo, J. (St. Louis Univ. Med. Sch., Mo.); Penchansky, L.; Monteleone, P. L.; Thompson, J. *J. Pediatr.* 86(1):98-102; 1975.

A newborn infant with bilateral Wilms' tumor, accompanied by striking Potter's facies and a previously unreported B-C translocation association is reported. The one-day-old white male had multiple congenital anomalies and normal initial hematologic and urine analysis values. An initial i.v. pyelogram revealed bilateral renal enlargement with marked distortion of the pelvocalyceal collecting systems. A subsequent i.v. pyelogram at three weeks showed severe hydronephrosis of the left kidney. A large unresectable mass filling the pelvocalyceal collecting system of the left kidney was revealed at laporatomy. It was diagnosed as bilateral Wilms' tumor; this was confirmed from later routine tissue examination. There was subsequent gastrointestinal hemorrhage at the ninth postoperative day; the child died at five weeks of age. The pathologic changes, limited to the kidneys, included an enlarged left kidney, a hemorrhagic mass occupying the entire upper renal pole and midportion of the renal parenchyma, and polypoid projections into the pelvis. The mass was edematous, papillomatous, and focally necrotic. The tumor was composed of islets of hematoxophilic epithelial tissue with scanty cytoplasm separated by myxomatous or fibroblastic tissue. Peripheral lymphocytes revealed the modal chromosome number of 46, with an increase in length of the long arm of one of the B group chromosomes and a deletion of portions of the long arms of one of the C group chromosomes. This was interpreted as a translocation between the two chromosomes. The author acknowledges the paucity of data suggesting consistent chromosome abnormalities in association with Potter's facies, and suggests the patient may represent either an isolated combination of such chromosomal abnormalities of Wilms' tumor, or an example of a more common association.

1040 AN EPENDYMOBLASTOMA OF THE PONS. (Eng.) Queiroz, L. S. (Dept. of Pathology, Campinas State Univ., Campinas, Brazil); Lopes de Faria, J.; Cruz Neto, J. N. *J. Pathol.* 115(4):207-210; 1975.

1041 CARCINOID TUMOUR AND CROHN'S DISEASE. (Eng.) Tehrani, M. A. (West. Infirm., Glasgow, Scotland); Carfrae, D. C. *Br. J. Clin. Pract.* 29(5):123-124; 1975.

1042 MALIGNANT CHONDROID SYRINGOMA OF THE THIGH. REPORT OF A CASE WITH ELECTRON MICROSCOPY OF THE TUMOUR. (Eng.) Webb, J. N. (Western General Hosp., Edinburgh, Scotland); Stott, W. G. *J. Pathol.* 116(1):43-46; 1975.

1043 MALIGNANT DEGENERATION OF A CHONDROMYXOID FIBROMA IN A CHILD. (Eng.) Sehayik, S. (Montreal Children's Hosp., 2300 Tupper St., Montréal, Quebec H3H 1P3, Canada); Rosman*, M. A. *Can. J. Surg.* 18(4):354-360; 1975.

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1100 HISTOCHEMISTRY OF REDUCTION-OXIDATION ENZYMES IN THE CELLS OF NEUROECTODERMAL TUMORS. (Rus.) Khominskii, B. S. (Dept. Pathomorphology, Kiev Sci. Res. Inst. Neurosurgery, Kiev, USSR). *Arkh. Patol.* 37(4):10-17; 1975.

1101 CHANGES IN FINE STRUCTURE ACCOMPANYING ESTROGEN-INDUCED TUMORIGENESIS OF LEYDIG CELLS IN THE MOUSE TESTIS. (Eng.) Kurland, G. (Temple Univ. Sch. of Medicine, 3420 N. Broad St., Philadelphia, Pa. 19140); Christensen, A. K.; Huseby, R. A. *Cancer Res.* 35(7):1671-1686; 1975.

1102 EXPERIMENTS AND THEORETICAL CELL KINETIC CALCULATIONS ON THE PROBLEM OF *IN VIVO* SYNCHRONIZATION WITH VINCRISTINE IN L 1210 ASCITES TUMOR CELLS AND CRYPT CELLS OF THE MOUSE. (Ger.) Jellinghaus, W. (Inst. f. Med. Strahlenkunde, Univ. Wurzburg, D-8700 Wurzburg, Versbacher Landstrasse 5, West Germany); Maidhof, R.; Schultze, B.; Maurer, W. *Z. Krebsforsch.* 84(2):161-176; 1975.

1103 GARDNER'S SYNDROME. (Eng.) Halse, A. (Univ. Hosp., 18 Tagensvej, DK-2200 Copenhagen N, Denmark); Roed-Petersen*, B.; Lund, K. *J. Oral Surg.* 33(9):673-675; 1975.

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* (Immun): 892, 913, 978, 982, 983, 988, 996
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1104 CONTRIBUTIONS OF CANCER REGISTRIES TO EPIDEMIOLOGICAL RESEARCH. (Eng.) Clemmesen, J. (No affiliation). *Recent Results Cancer Res.* 50: 119-131; 1975.

Contributions of cancer registries to epidemiologic research are presented. Cancer registrations for the study of rare neoplasms were found to offer the best conditions for information analysis and epidemiologic application (e.g. geographic mapping of the distribution of cases of tumors of the jaw and testes), but only a few registries have been established under these circumstances. The Danish cancer registry, in 1942, studied the social distribution of lung cancer and confirmed previous observations of the rise in lung cancer among males in Copenhagen. The registry also studied the rising incidence of urinary bladder neoplasms in Copenhagen men and found that the onset and end of urinary bladder neoplasms occurs 9.3 yr later than that for lung cancer at age 47 and 10.5 later at age 64. Registries for epidemiologic results were not as successful for cancers of the large intestine, rectum, and stomach; it is easier to interpret data from registries for cancer that are increasing in incidence rather than from registries for established cancers. Thus, epidemiologic results vary with the type of neoplasm in question. Cancer registries have made it possible to follow a neoplasm from the time it was a rarity to the present. Contributions of cancer registries have also been useful in other fields. It is because of these advantages that the author suggests that cancer registries be developed under as widely varying conditions as possible and used in conjuction with other methods for collecting and disseminating information.

1105 THE CANCER REGISTRY OF CALI COLOMBIA -- EPIDEMIOLOGIC STUDIES OF GASTRIC CANCER. (Eng.) Correa, P. (No affiliation); Bolaños, O.; García, F. T.; Gordillo, G.; Duque, E.; Cuello, C. *Recent Results Cancer Res.* 50:155-169; 1975.

The general operation of the registry, and work done in the area of gastrointestinal tumors were described. The data reported were obtained from the city of Cali, which has four well-demarcated boundaries; the 1964 national census set the population at 638,211. Employing medical students in interviewing and data collection, the studies began with the registry findings and progressed to other special clinical, pathologic, and field studies. Figures indicate the incidence rate of gastric cancer in Cali males was 57.5 per 100,000, second only to Japan. Further analysis of the figures considered place of birth and population of origin. Available histologic material was reviewed and classified by the system of Jarvi and Lauren, and indicated an excess of all histologic types for the immigrants from the rural high-altitude South, especially of the intestinal type. In attempting to link incidence data with the prevalence of lesions (potential cancer precursors), gastric specimens were obtained and special attention was given to intestinal metaplasia of the gastric mucosa. Prevalence figures for each migrant group were determined, and a clear gradient was observed indicating areas representing the extremes of gastric cancer risk. In-depth studies of such areas aimed at the identification of factors associated with intestinal metaplasia or other precancerous conditions, were undertaken by examining populations with drastically different prevalence rates. In addition to a special questionnaire, biochemical tests, gastroacidogram, gastroscopy and gastric biopsy were employed. Symptoms showing the greatest discrimination between the two populations included sensations of "fullness" in the epigastric area, vomiting, nausea, pyrosis, and pain; likewise, a greater prevalence of lesions was found in the high-risk areas, while biochemical tests lacked any discrimination. Limited environmental studies indicated that in areas of highest risk, the water supplies have a high nitrate content, most especially in dug-wells and subterraneous fountains. Cancer registration should be encouraged in developing countries, not only to provide information about the local situation but also to study trends in other countries; cancer incidence in western countries may contrast with incidence in developing countries.

1106 CANCER INCIDENCE IN RELATION TO FLUORIDE LEVEL IN WATER SUPPLIES. (Eng.) Kinlen, L. (Dep. Regius Professor Med., Oxford Univ., England). *Br. Dent. J.* 138(6):221-224; 1975.

The incidence of thyroid, kidney, stomach, esophagus, colon, rectum, bladder, bone, and breast cancers in areas of England and Wales with high fluoride levels in the water (natural and artificial) was compared with that in control areas having low fluoride levels. Cancer registration data from fluoridated and control areas of the U.S.A., Holland, and New Zealand were also examined. Information on fluoride levels in water was obtained from the water board of each local district. Data from districts with high fluoride levels ($\geq$ 1 ppm) was matched with a nearby district, similar in size, with low fluoride level ($\leq$ 0.2 ppm). No relation was found between fluoride levels and cancer incidence; the incidence of cancer of any organ was no greater in areas in which water had high fluoride content than in control areas.

1107 SOLVENT EXPOSURE AND LEUKEMIA AMONG RUBBER WORKERS: AN EPIDEMIOLOGIC STUDY. (Eng.) McMichael, A. J. (Sch. Public Health, Univ. North Carolina, Chapel Hill); Spirtas, R.; Kupper, L. L.; Gamble, J. F. *J. Occup. Med.* 17(4):234-239; 1975.

An epidemiologic study (consisting of cohort mortality and case control studies) was conducted by the School of Public Health and the Univ. of North Carolina. A cohort of 6,678 male, hourly rubber workers at a plant in Akron, Ohio was identified. Workers (40-84 yr of age) had been employed an average of 25 yr. Mortality data were obtained from the company's life insurance records with only 1% of the persons being lost to follow-up. Cancer mortality was significantly elevated among rubber workers when compared to a standard population, particularly cancers of the stomach, prostate, and lymphatic and hematopoietic systems (including the leukemias). In the 40-64 age group, there was a 3-fold excess of deaths due to leukemia and in the 40-84 age group, a 2-fold excess for lymphoid tissue

cancer (lymphosarcomas, reticulum cell carcinomas, and Hodgkin's disease). The case control study included 88 hourly workers (retired and active) dying between 1964 and 1973, whose death certificate referred to cancer of the lymphatic or hematopoietic systems. Controls consisted of rubber workers who had not died of this type of cancer. This analysis revealed a positive association between solvent exposure and lymphatic leukemia. The death risk from lymphatic leukemia is 7:1 for workers in high-solvent-exposure jobs as compared to other workers and 2:1 for medium or light solvent-exposure jobs. No further conclusions can be drawn; information from other rubber companies and knowledge of the particular solvents in use must be investigated.

1108 AN EPIDEMIOLOGICAL STUDY OF EXPOSURE TO COAL TAR PITCH VOLATILES AMONG COKE OVEN WORKERS. (Eng.) Mazumdar, S. (Dep. Biostatistics, Univ. Pittsburgh, Pa.); Redmond, C.; Sollecito, W.; Sussman, N. *J. Air Pollut. Control Assoc.* 25(4): 382-389; 1975.

A study was conducted to relate total work history and coal tar pitch volatile (CTPV) exposure to lung cancer mortality observed among coke oven workers. Exposure data were taken from a study conducted by the Pa. Dept. of Health. Mortality data were based on a long-term study of steel-workers conducted by the Dept. of Biostatistics, Univ. of Pittsburgh. Coke oven jobs were categorized into three different work areas in terms of exposure to CTPV: topside oven workers receiving maximum exposure; side oven 1 workers receiving intermediate exposure; and side oven 2 workers, who were exposed for only short periods of time or who worked at a distance from the ovens (receiving minimal exposure). A cumulative exposure index was then developed to investigate the dose-response relationship of exposure and cancer mortality. Measured levels of CTPV were 2 to 3 times higher for topside workers than for side oven workers (average values were 3.15 mg/m^3, 1.99 mg/m^3, and 0.88 mg/m^3 for topside, side oven 1 and side oven 2 workers, resp.). The level of exposure and the length of time of exposure were found to be related to the development of cancer, particularly lung cancer; the risk of cancer development was 2-fold in age groups 55 and over. The findings of this report suggest that the recommended threshold limited value to CTPV exposure should not exceed 0.2 mg/m^3 for an average period of 30 yr. A value higher than this would increase the risk of death from lung cancer. However, further measurements and analyses must be made as this study did not include any data on the smoking habits of steel workers, which may influence mortality values.

1109 MATHEMATICAL DESCRIPTION AND ANALYSIS OF CELL CYCLE KINETICS AND THE APPLICATION TO EHRLICH ASCITES TUMOR. (Eng.) Kim, M. (Sch. Electr. Eng., Cornell Univ., Ithaca, N.Y.); Bahrami, K.; Woo, K. B. *Theor. Biol.* 50(2):437-459; 1975.

The linear and nonlinear aspects of the dynamics of cell cycle kinetics of cell populations were studied *via* a mathematical model. The dynamics were represented by nonlinear difference equations, and the characteristics were analyzed by application to Ehrlich ascites tumor (EAT). The model used for the description of cell cycle kinetics considered a total population consisting of two groups of cells, proliferating and nondividing. A discrete time model was employed, cell size departments were defined, and a Gaussian distribution was assumed. Investigations of the pattern of cellular proliferation of the EAT cells in the peritoneal cavity have shown that growth is initially very rapid, due to cellular proliferation, followed by a phase of progressive deceleration. Growth parameters considered included mean generation time, changes in mean size, mean DNA and RNA contents of the cells, and changes in cell size distribution. The dependence of the mean generation time of EAT cells on the tumor age was investigated, indicating a progressive increase in the relative number of larger cells, with cell volume illustrating two peaks. The transition of proliferating cells to the resting states and its effects on population growth were then considered, and the simulated cell age distribution was presented. Time course behavior of cell size distribution was determined, and changes in the mean size and mean volume of EAT cell population was studied. The simulation illustrated an increase in the relative number of larger cells with aging of the tumor, comparing favorably with experimental results. The time course behavior of cellular DNA distribution predicted the mean DNA content/cell increases monotonically with aging of the tumor. From the computer simulation of the model, it was concluded that the retarding of the rate of growth of EAT population is due to a combination of several factors; these include prolongation of mean generation time of proliferating cells, the transition of proliferating cells to the resting states, and the higher rate of cell death for the older tumor. From the general quantitative model it was thus concluded that the process of transition to resting states depends on the size of the population of the tumor, which in turn depends on the change in growth media and on the increase in some type of growth inhibitor brought about by tumor aging.

1110 THE RESPONSE OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES TO PHYTOHEMAGGLUTININ: DETERMINATION OF CELL NUMBERS. (Eng.) Stewart, C. C. (Washington Univ. Sch. Med., St. Louis, Mo.); Cramer, S. F.; Steward, P. G. *Cell Immunol.* 16(2): 237-250; 1975.

A description of human peripheral blood lymphocytes in terms of cell number, morphology, and tritiated thymidine incorporation as a function of time following stimulation with PHA is presented. Lymphocyte cultures obtained from seven healthy donors of both sexes from 20-40 yr old were used in 21 separate experiments. Lymphocytes were incubated with 100 mg PHA/5.0 ml culture medium. An improved pronase-cetrimide counting technique was used to determine the number of viable lymphocytes as a function of time. Nuclei volume changes obtained after cetrimide (10 ml) treatment were quantified with a curve-fitting computer program. Lymphocytes exhibited an induction phase (0-2 days) characterized by de-

creased cellularity and increased nuclear volume, an exponential proliferation (2-5 days) with a continued increase in the number of cells with large nuclear volume, and a lysis phase (5-14 days) characterized by decreased cellularity and nuclear volume. This suggests that the ratio of cell number in culture to the volume of culture medium is crucial for optimal transformation and proliferation; better responses were produced with 10^5 cells/ml than with 10^6 cells/ml, due to nutrient depletion.

1111 A COMPUTER SIMULATION PROGRAM FOR THE STUDY OF CELLULAR GROWTH KINETICS AND ITS APPLICATION TO THE ANALYSIS OF HUMAN LYMPHOMA CELLS *IN VITRO*. (Eng.) Donaghey, C. E. (Dept. Syst. Ind. Eng., Univ. Houston, Tex.); Drewinko, B. *Comput. Biomed. Res.* 8(2):118-128; 1975.

A cell kinetics computer simulation program (CELLSIM) employing simple language, principles, and flexibility was described. Instructing the program involves designating the cell population, cell flow, time span in each state, proliferation rate and initial cell inoculum. All memory in CELLSIM is dynamically allocated, permitting any necessary reassignment. CELLISM was used to model growth kinetics of an established human immunoglobin-producing cell line derived from the tumorous lymph nodes of a patient with lymphocytic lymphoma; the parameters assessed by the model gave values almost identical to those determined experimentally. Apparently useful in manipulating growth kinetics parameters in the correlation of cell cycle-related effects executed by chemotherapeutic agents, the program is currently utilized in predicting the survival behavior of populations of the T_1 cell line, following treatment with drugs. The authors acknowledge several deficiencies and arbitrary assumptions, yet emphasize the built-in flexibility capable of incorporating or modifying assumptions concurrent with available experimental evidence.

1112 SMOKING HABITS AND LUNG CANCER EPIDEMIOLOGY IN AUSTRIA. CHANGES IN LUNG CANCER MORTALITY IN MEN? (Ger.) Friedl, H. P. (Institut fur Krebsforschung der Universitat Wien, Wien, Austria); Karrer, K.; Wrba, H. *Oesterr. Z. Onkol.* 2(2/3):59-63; 1975.

1113 CONCERNING THE DYNAMICS OF OVARIAN TUMOR INCIDENCE. (Rus.) Dzardemov, A. A. (Kazakh Res. Inst. Oncol. Radiol., Kazakh SSR Ministry Health, USSR); Sabyrov, G. S. *Vopr. Onkol.* 21(3):40-44; 1975.

1114 CHANGES IN CELL PROLIFERATION KINETICS OCCURRING DURING THE LIFE HISTORY OF MONOLAYER CULTURES OF A MOUSE TUMOR CELL LINE. (Eng.) Twentyman, P. R. (Middlesex Hosp. Med. Sch., London, England); Watson, J. V.; Bleehen, N. M.; Rowles, P. M. *Cell Tissue Kinet.* 8(1):41-50; 1975.

1115 EXTENSIONS OF THE RELATIVE RISK CONCEPT. (Eng.) Bertell, H. R. (Roswell Park Mem. Inst., Buffalo, N.Y.). *Experientia* 31(1): 1-10; 1975.

1116 CYTOKINETIC CONSIDERATIONS OF MURINE NEUROBLASTOMA (C1300) AS A SCREENING MODEL FOR THE CHILDHOOD DISEASE. (Eng.) Finklestei J. Z. (Harbor General Hosp., 1000 West Carson St., Torrance, Calif. 90509); Weiner, J. *Eur. J. Cancer* 11(3):175-179; 1975.

1117 PROLIFERATION AND COLONY-FORMING ABILITY OF PERITONEAL EXUDATE CELLS IN LIQUID CULTURE. (Eng.) Stewart, C. C. (Washington Univ. Sch. of Medicine, St. Louis, Mo. 63110); Lin, H.-S.; Adles, C. *J. Exp. Med.* 141(5):1114-1132; 1975.

1118 PROLIFERATIVE PATTERNS IN COLONIC MUCOSA IN FAMILIAL POLYPOSIS. (Eng.) Deschner, E. E. (Mem.-Sloan-Kettering Cancer Cent., New York, N.Y.); Lipkin, M. *Cancer* 35(2):413-418; 1975.

1119 EARLY DETECTION OF LUNG CANCER IN HIGH RISK POPULATIONS. (Eng.) Brooks, S. M. (Coll. Med., Univ. Cincinnati, Ohio). *J. Occup. Med.* 17(1):19-22; 1975.

1120 COALWORKER'S PNEUMOCONIOSIS AND CARCINOMA OF THE LUNG. (Eng.) McLintock, J. S. (Natl. Coal Board, London, England). *Lancet* 1(7900): 224-225; 1975.

1121 THE CONCENTRATIONS OF BENZO(a)PYRENE IN BOMBAY. (Eng.) Rao, A. M. M. (Div. Radiological Protection, Bhabha Atomic Res. Centre, Trombay, Bombay-400 085, India); Vohra, K. G. *Atmos. Environ.* 9(4):403-408; 1975.

1122 HODGKIN'S DISEASE MORTALITY AMONG PHYSICIANS. (Eng.) Matanoski, G. M. (Sch. Hygiene and Public Health, Johns Hopkins Univ., 615 North Wolfe St., Baltimore, Md. 21205); Sartwell, P. E.; Elliott, E. A. *Lancet* 1(7912):926-927; 1975.

1123 CARCINOGENESIS DUE TO MUSTARD GAS EXPOSURE IN MAN. (Ger.) Weiss, A. (Innere Abteilung des Krankenhauses Elim, Hamburg, West Germany); Weiss, B. *Dtsch. Med. Wochenschr.* 100(17):919-923; 1975.

1124 THE RECURRENT GROWTH OF ASCITE HEPATOMA 22A. (Rus.) Beliaeva, I. D. (Inst. Biological and Medical Chemistry Acad. Medical Sciences U.S.S.R., Moscow, U.S.S.R.); Ivleva, T. S. *Tsitologiia* 17(6):688-693; 1975.

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* (Chem): 721
* (Phys): 792
* (Path): 1003, 1004, 1102

1125 CHORIOALLANTOIC MEMBRANE HETEROTRANSPLANTATION OF HUMAN BRAIN TUMORS. (Eng.)
Vogel, H. B. (Jefferson Med. Coll., Philadelphia, Pa.); Berry, R. G. *Int. J. Cancer* 15(3):401-408; 1975.

The results of transplantation of human brain cell tumor onto the chorioallantoic membrane (CAM) of the chick egg are reported. White Leghorn eggs, 8-12 days old, served as recipients. A tumor 2 mm in diameter was placed over a prominent vessel in the CAM. Specimens were excised from the CAM at 19 days of age. Seventy-four percent of the eggs survived the procedure and 40% of the transplants were viable after one week. Best growth occurred with benign, non-neuroectodermal tumors. The ability to effect serial passage was limited to two or three generations. The degree of necrosis increased progressively with serial heterotransplants. The degree of necrosis depended on the size of the fragments, the degree of tumor envelopment and the extent of tumor revascularization by CAM vessels. Microscopically, the first generation transplants retained resemblance to parent tumors. Later generations retained nuclear character but were not found in characteristic patterns. This technique cannot be compared with tissue culture because the egg is considered a laboratory animal. The biological interaction between foreign tissue fragments and chick embryos remains to be explained and may be a viable method in studying tumor-host relationships.

1126 CLASSIFICATION AND BIOLOGICAL NATURE OF ESTABLISHED HUMAN HEMATOPOIETIC CELL LINES. (Eng.) Nilsson, K. (Dep. Pathol., Univ. Uppsala, Sweden); Pontén, J. *Int. J. Cancer* 15(2):321-341; 1975.

Over 200 established human hematopoietic cell lines of normal and malignant origin were studied by morphological and functional parameters. Four lines were identified. Lymphoblastoid cell lines, derived from normal and neoplastic hematopoietic tissue, were characterized by the wide morphologic flexibility of individual cells, constant association with Epstein-Barr virus (EBV), polyclonal derivation, differentiation for immunoglobulin (Ig) production, and a diploid karyotype. Lymphoma cell lines were established at a high frequency from Burkitt's lymphoma, rarely from other types of lymphoma, and never from patients without malignancy or with non-lymphoma malignancies. Important characteristics were constant morphology, monoclonal derivation, common association with EBV, variability in the expression of Ig synthesis, and aneuploidy. Myeloma cell lines were only rarely obtained from patients with myeloma. The basis for classification is the production of Ig identical to the myeloma protein *in vitro*. Other features were plasma cell morphology, absence of EBV and aneuploidy. Important characteristics of the leukemia cell line were: a typical surface ultrastructure, absence of EBV, and absence of Ig production. It is concluded that EBV-carrying lymphoblastoid lines can be obtained from non-neoplastic precursor cells from healthy and from diseased individuals. Lymphoma, myeloma, and leukemia lines are only obtained from the respective neoplastic tissue and only at a low frequency.

1127 RELATIONSHIP BETWEEN FIBRINOLYSIS OF CULTURED CELLS AND MALIGNANCY. (Eng.) Laug, W. E. (Child. Hosp. Los Angeles, Calif.); Jones, P. A.; Benedict, W. F. *J. Natl. Cancer Inst.* 54(1): 173-179; 1975.

Cells cultured from various human and nonhuman malignant and normal tissues and mammalian cells transformed *in vitro* were examined for their ability to induce fibrinolysis in fetal calf, dog, and human sera. Cells (500,000) were added to a ^{125}I-fibrin-coated Petri dish containing 5 ml medium plus serum being tested. Aliquots of supernatant were taken at 3, 6, 12, 24, 48, and 72 hr to measure the radioactivity released. Except for kidney and lung tissue, cells cultured from normal mammalian tissues show little fibrinolytic activity. Most cell types derived from neoplastic tissue or those chemically transformed *in vitro* possess high levels of fibrinolytic activity (greater than 20% fibrinolysis after 24 hr). Correlations were drawn between the ability of cells to grow in soft agar, the ability to form tumors in immunosuppressed animals, and the expression of fibrinolytic activity. Fibrinolytic activity can be rapidly and easily detected in regular tissue culture medium supplemented with sera from three different species. Thus, it is concluded that fibrinolytic activity may be a useful criterion for malignancy and transformation in cell culture.

1128 MAMMALIAN CELL FUSION. VI. REGULATION OF MITOSIS IN BINUCLEATE HELA CELLS. (Eng.) Rao, P. N. (Univ. Texas M. D. Anderson Hosp. Tumor Inst., Houston); Hittelman, W. N.; Wilson, B. A. *Exp. Cell Res.* 90(1):40-46; 1975.

The inhibitory effect of the S phase component in S/G2 heterophasic binucleate cells on the progression of the G2 nucleus to mitosis was investigated. HeLa cells were grown as a suspension culture at 37 C. Cells were synchronized by the excess thymidine (2.5 mM) double block technique. In two different cell fusion experiments a synchronized population of HeLa cells, prelabeled with ^{3}H-TdR, was fused with an unlabeled one using inactivated Sendai virus. In the first experiment, HeLa cells in early G2 phase which were exposed to either 4 C, cycloheximide, actinomycin D or X-irradiation were fused separately with untreated and more advanced G2 cells. A comparison of the rates of mitotic accumulation (in the presence of Colcemid) for the various classes of mono- and binucleate cells revealed that the hybrid cells were intermediate between those of the advanced and the retarded parental types, indicating that the chromosome condensing factors of the advanced component were diluted as a result of such fusion. The manner in which the retarding effects of actinomycin D and cycloheximide were reversed in the hybrid cells suggests that proteins had a major role as chromosome condensing factors in the G2 mitotic transition. In the second experiment, when S phase HeLa cells were fused with those in

G2, the resulting heterophasic (S/G2) binucleate cells reached mitosis at about the same time as the homophasic (S/S) cells of the lagging parent, indicating a complete dominance of the S over the G2 with regard to their progress towards mitosis. The addition of Mg^{++} (2×10^{-2} M of $MgCl_2$) to the medium helped the G2 nuclei to enter mitosis asynchronously, which consequently induced premature chromosome condensation in the S phase component. These data suggest that in the heterophasic (S/G2) binucleate cells the S phase component causes decondensation of the G2 chromatin, thus blocking it from entering mitosis. This effect, which did not appear to be dose-dependent, could be neutralized and the G2 nuclei relieved from this repression by an external supply of Mg^{++} ions.

1129 INHIBITION OF TUMOR ANGIOGENESIS MEDIATED BY CARTILAGE. (Eng.) Brem, H. (Harvard Med. Sch., Boston, Mass.); Folkman, J. *J. Exp. Med.* 141(2):427-439; 1975.

The ability of neonatal rabbit cartilage to inhibit the capillary proliferation induced by solid tumors was studied. V2 carcinoma was implanted in a corneal pocket of male New Zealand rabbits, and a tiny piece of neonatal New Zealand rabbit cartilage was placed between the tumor and the limbal edge of the cornea; lyophilized, boiled, reconstituted neonatal cartilage, neonatal cornea, and neonatal bone were substituted for the neonatal cartilage in the eyes of control rabbits. In seven-day-old White Leghorn embryos, Walker carcinosarcoma or a crude tumor-angiogenesis-factor (TAF) was placed on the chorioallantoic membrane (CAM) and neonatal cartilage was implanted nearby; in control eggs, lyophilized, boiled cartilage was substituted for untreated cartilage. In 15/53 rabbit corneas containing tumor with cartilage, vascularization of the tumors was completely inhibited; these nonvascularized tumors appeared to undergo immunological rejection. In the remaining 38 tumors, vascularization eventually occurred, but it was delayed by an average of three weeks; once the tumor was vascularized, rapid exponential growth ensued and the cartilage was completely engulfed by tumor. Normal tumor neovascularization was seen in all tumors implanted with boiled cartilage, neonatal cornea, or neonatal bone. In 23 of the 28 chick embryos in which neonatal cartilage was implanted with Walker carcinosarcoma, there was an avascular zone of 1-2 mm diameter around the cartilage; a similar zone was observed in 9/11 CAM when cartilage was implanted with crude TAF. Boiled cartilage had no inhibitory effect on tumor neovascularization. The results suggest that the cartilage inhibitor does not antagonize the TAF, but that it inhibits capillary proliferation directly. With further purification, this material may prove useful as a means of maintaining tumor dormancy by "antiangiogenesis".

1130 HISTONE ABNORMALITIES IN ADULT ACUTE LEUKEMIAS. (Eng.) Kass, L. (Simpson Mem. Inst., Univ. Michigan, Ann Arbor). *Blood* 45(4):477-484; 1975.

Differences in the electrophoretic patterns of lysine-rich and arginine-rich histones subjected to electrophoresis in polyacrylamide gel were described. The histones were extracted from 20 ml of heparinized peripheral venous blood from 14 leukemia patients. In addition to the electrophoresis, photomicrographs, non-specific and specific esterase reactions, the PAS test for glycogen was performed. The patterns of normal, monocytic histones (three arginine bands [3A], three lysine bands [3L]) resembled those found in acute histomonocytic leukemia (Schilling type, 3A, 3L); therefore acute histomonocytic leukemia is likely a monocytic rather than a myeloblastic disorder. The histones from leukemic blasts in acute myelomonocytic leukemia (Naegli type, 6L, 8A) were similar to those in both acute myeloblastic leukemia (6L, 7A) and chronic granulocytic leukemia (6L, 9A) and appeared to be related at the nucleoprotein level. Thus, myelomonocytic leukemia may be a cytologic variant of acute myeloblastic leukemia. Histones from chronic granulocytic leukemia extracted from myelocytes and progranulocytes showed an electrophoretic pattern similar to myeloblasts, suggesting common histones. The differences and similarities in the electrophoretic patterns of histones might be used to distinguish between acute myelomonocytic, myeloblastic and histomonocytic leukemia, supporting and/or replacing conventional tests.

1131 INTERACTING POPULATIONS AFFECTING PROLIFERATION OF LEUKEMIC CELLS IN CULTURE. (Eng.) Aye, M. T. (Inst. Med., Univ. Toronto, Canada); Till, J. E.; McCulloch, E. A. *Blood* 45(4):485-494; 1975.

Leukocytes of three acute leukemic patients were analyzed by velocity sedimentation, growth curves, chromosomal analysis, and analysis by mixing. These analyses were performed after preliminary characterization of the leukocyte responses to a leukocytic-conditioned medium (LCM) and to phytohemagglutinin (PHA). As expected, the majority of the lymphocytes would not respond to the mitogens, but PHA-responsive (PHA-r) cells formed aneuploid metaphases in PHA cultures and hence belong to the same clone as the LCM-responsive (LCM-r) cells. Velocity sedimentation support the view that two and possibly three subpopulations were present. The mixing experiments showed functional interaction between the PHA-r and the LCM-r cells; some irradiated PHA-r cells from the fraction pooled on their LCM response capacity, yet when PHA was added, the initiation of incorporation occured. Hence, leukemic peripheral blood contains cells that respond to PHA by proliferation and also cells that respond by stimulating the proliferation of cells in a separate population this constitute evidence for heterogeneity among the cells of acute myeloblastic leukemia patients.

1132 CYCLIC AMP AND CYCLIC GMP CONCENTRATIONS IN SERUM- AND DENSITY-RESTRICTED FIBROBLAST CULTURES. *(Eng.)* Moens, W. (Universite Libre de Bruxelles, Departement de Biologie Moleculaire, 67, rue des Chevaux, 1640 Rhode St. Genese, Belgium); Vokaer, A.; Kram, R. *Proc. Natl. Acad. Sci. USA* 72(3):1063-1067; 1975.

The concentrations of 3':5'-cyclic AMP (cAMP) and 3':5'-cyclic GMP (cGMP) in serum- and density-inhibited cultures of 3T3 cells, SV40-transformed 3T3 cells (SV3T3-101), and four revertant lines derived from SV101 were studied. Revertant clones F1SV101 and BuSV2 were density dependent, and the serum-dependent clones LsSV2 and AγSV5 were unable to grow at low serum concentrations (1%) and in gamma-depleted 10% calf serum, respectively. The intracellular cAMP and cGMP levels were assayed in cultures growing in medium containing 10% or 0.5% calf serum and in cultures grown to confluence in 10% serum. The cAMP levels in the growing SV101 cultures were about 1/2 that observed in the 3T3 cultures and the cGMP levels in the transformed cultures were about twice those in the untransformed cells. The cAMP pattern of Aγ SV5 was similar to that of SV101, while the cAMP patterns in the other revertants were similar to that of 3T3. The cGMP concentrations in the serum-dependent revertants were similar to that of 3T3, while those in the density-dependent revertants were similar to that of SV101. Density-dependent inhibition of growth was correlated with reduced cGMP levels in 3T3 and the four revertants, while the levels in SV101 were only slightly decreased. The cAMP levels decreased slightly in all cultures at confluence. In medium containing 0.5% serum, the serum-dependent lines showed a greater increase in intracellular cAMP than did the serum-insensitive lines, while the cGMP were greatly reduced in all except SV3T3 by SV101. Serum readdition to all serum-dependent lines was followed by a rapid decrease in cAMP and an increase in cGMP. The magnitude of these responses was decreased in SV101 and the density revertants. The data suggest that decreased cGMP concentrations might specifically mediate sensitivity to density-dependent inhibition of growth and that serum- and density-dependence are distinct.

1133 CYCLIC AMP, MEMBRANE TRANSPORT AND CELL DIVISION. I. EFFECTS OF VARIOUS CHEMICALS ON CYCLIC AMP LEVELS AND RATE OF TRANSPORT OF NUCLEOSIDES, HYPOXANTHINE AND DEOXYGLUCOSE IN SEVERAL LINES OF CULTURES CELLS. *(Eng.)* Sheppard, J. R. (Dight Inst. Hum. Genet., Univ. Minnesota, Minneapolis); Plagemann, P. G. W. *J. Cell Physiol.* 85 (2):163-172; 1975.

The relationship between the cellular cyclic AMP level and uridine, thymidine, hypoxanthine, and deoxyglucose transport was studied in Novikoff rat hepatoma cells, Polyoma virus-transformed 3T3 cells, 3T6, C6 glioma cells and mouse L cells. Cell suspensions or plate cultures were incubated with papaverine, prostaglandin E_1, isoproterenol, Persantin, Cytochalasin B, or dibutyryl cyclic AMP and ^{3}H-labeled substrate. Papaverine-, prostaglandin E_1-, and isoproterenol-induced changes in the intracellular cyclic AMP levels within the test cultures were not correlated with the inhibition of uridine, hypoxanthine, or deoxyglucose transport. Chemically-induced inhibitions in the transport rates occurred in most cell lines in the absence of any measurable change in the intracellular cyclic AMP concentration. Furthermore, treatment of several cell lines with 1 mM dibutyryl cyclic AMP had no immediate effect on the transport of uridine, thymidine, or deoxyglucose; however, the transport capacity of the cells for uridine and thymidine, but not that for deoxyglucose, decreased progressively with time. Although the uridine transport system of the 3T3-derived cells and the hypoxanthine transport system of the L cells exhibited high degrees of resistance to inhibition by the various chemicals tested, deoxyglucose transport was inhibited to about the same extent in all cell lines. The results suggest that the competitive inhibitions may be due to the binding of the inhibitor to a binding site different from the substrate binding site, but located in close proximity to the latter. Alternatively, it is possible that the inhibitors affect transport processes by competitively binding to the substrate binding sites and that the binding proteins of different cell lines exhibit different affinities for the inhibitors. The data do not support the hypothesis that cellular cyclic AMP levels and cellular transport systems are related and that they cooperatively control cell division.

1134 DETECTION OF NERVE GROWTH FACTOR BINDING SITES ON NEUROBLASTOMA CELLS BY ROSETTE FORMATION. (Eng.) Revoltella, R. (Laboratorio di Biologia Cellulare, Consiglio Nazionale delle Ricerche, Roma, Italy); Bosman, C.; Bertolini, L. *Cancer Res.* 35(4):890-895; 1975.

A method for detecting nerve growth factor (NGF)-specific receptor sites by measuring the capacity of neuroblastoma cells to bind sheep red blood cells (SRBC) passively coated with NGF, through the formation of rosettes, was investigated. NGF is a protein capable of producing rapid differentiation of normal sympathetic cells *in vivo* and *in vitro*. Neuroblastoma cells were synchronized and tested for rosette formation (cells with five or more adherent red cells) during late G_1 phase of the cell cycle. Rosettes formed rapidly, optimally at physiological pH, were stable and were largely dependent on the number of NGF-coated SRBC and on the number of NGF molecules coated onto each erythrocyte. Cytotoxic drugs, noxious agents and metabolic inhibitors were usually ineffective in inhibiting rosette formation, except for proteolytic enzymes; hence surface structures were most likely of protein nature. Binding of NGF did not depend on divalent cations and proceeded at low temperatures. Cell fixation did not affect appropriate receptor sites. The specificity of the NGF receptor cells was questioned and the existence of common receptor sites on all cells was proposed. Cell site reactivity was found to be a function of the experimental conditions, or of the fact that some sites may exist in common with other cells, while other sites are only on the surface of neuroblastoma and sympathetic cells. This suggests that neuroblastoma cells may be useful in the study of induction of differentiation by NGF.

1135 PHAGOCYTOSIS OF NERVE GROWTH FACTOR-COATED ERYTHROCYTES IN NEUROBLASTOMA ROSETTE-FORMING CELLS. (Eng.) Bosman, C. (Laboratorio di Biologia Cellulare, Consiglio Nazionale delle Richerche, Roma, Italy); Revoltella, R.; Bertolini, L. *Cancer Res.* 35(4):896-905; 1975.

Rosette formation and the sequence of cell membrane changes found in clone NB/1R murine C1300 neuroblastoma cells were studied following interaction with nerve growth factor (NGF)-coated sheep RBC. The neuroblastoma cells formed rosettes when they were synchronized in the G_1 phase of their growth cycle and incubated for 5 min at 2 C, pH 7.4 with NGF-coated, gluteraldehyde-fixed sheep RBC. The cells were examined by light and electron microscopy after the temperature was increased to 37 C. They underwent a rapid transformation characterized by microtubule formation, which occurred under the membrane surface close to points of contact with the attached RBC. The cell body then emitted cytoplasmic processes which surrounded the attached RBC and, after 20 min, the RBC were completely adherent to the neuroblastoma cell surface through broad and continuous areas of contact. Within 30-60 min, the RBC were completely phagocytized. Interiorization of RBC complexes to a neuroblastoma cell through the mediation of complement could be similarly induced after 20 min at 37 C. Pretreatment with colchicine (up to 10^{-5} M) or vinblastine (up to 10^{-4} M), which block microtubule formation, caused reduction in phagocytosis. Complete inhibition occurred only when the cells were pretreated with cytochalasin B, a strong inhibitor of microfilament contraction. The data support the hypothesis that tubulins, the major protein constituents of microtubules and the cytochalasin B-dependent contractile filamentous system, play an essential role in triggering membrane-bound RBC phagocytosis.

1136 CHANGES IN ADENYLATE ENERGY CHARGE IN EHRLICH ASCITES TUMOR CELLS DEPRIVED OF SERUM, GLUCOSE, OR AMINO ACIDS. (Eng.) Live, T. R. (Harvard Med. Sch., Boston, Mass.); Kaminskas, E. *J. Biol. Chem.* 250(5):1786-1789; 1975.

Changes in adenylate pools in Ehrlich ascites tumor cells and in the adenylate energy charge values under conditions of serum, glucose, or amino acid starvation are described. The possibility that serum factors may regulate protein synthetic rates by way of the adenylate energy charge was investigated. Concentrations of 5'-adenylates in cellular extracts were determined by polyethyleneimine-cellulose chromatography. The mean concentration of 5'-adenylates in cells growing in complete medium was 5.7 ± 0.2 nM/10^6 cells. Depriving cells of amino acids, glucose, and serum resulted in 24, 26, and 11% lower 5'-adenylate concentrations, resp. Protein synthesis rates were lower by 41 to 57% in serum-starved than in serum-grown cells. The adenylate energy charge values were 0.93 to 0.95 in the former and 0.80 to 0.85 in the latter. When cells were deprived of amino acids, protein synthetic rates declined 24 to 32% and the adenylate energy charge decreased to 0.59 to 0.74. When cells were incubated in glucose-free medium, protein synthetic rates decreased to 43 to 50% of cells incubated in complete medium. Their adenylate energy charge declined to 0.25 to 0.30. Supplementation of deprived cells with respective nutrients restored normal protein synthetic rates and adenylate energy charge values. Serum-deprived cells grown in depleted serum media do not increase their protein synthetic rate and their adenylate energy charge remains elevated. These results suggest that serum factors regulate protein synthetic rates by mechanisms other than those regulating the availability in cells of glucose or amino acids. The increased use of glucose and amino acids after addition of serum may be due to increased biosynthetic requirements.

1137 SERUM LEVELS OF N^2,N^2-DIMETHYLGUANOSINE AND PSEUDOURIDINE AS DETERMINED BY RADIOIMMUNOASSAY FOR PATIENTS WITH MALIGNANCY. (Eng.) Levine, L. (Dep. Biochem., Brandeis Univ., Waltham, Mass.); Waa'kes, T. P.; Stolbach, L. *J. Natl. Cancer Inst.* 54(2):341-343; 1975.

Sera of cancer patients were radioimmunoassayed for methylated nucleosides and pseudouridine. Serum was taken from randomly chosen cancer patients, primarily with breast cancer or leukemia. Most had active disease and some were receiving antitumor therapy. Several patients with tumors of unknown origin or brain tumors were also included. Control samples were taken from normal volunteers. To extract the nucleosides, serum was added to a methylalethanol mixture, the denatured protein filtered out, suspended in distilled water and dialyzed. The nucleosides were then converted to their ε-aminocaproate derivatives to increase the radioimmunoassay sensitivity. Elevated levels above two standard deviations of the normal average (3.6 ng/ml for N^2,N^2-dimethylguanosine and 0.42 μg/ml for pseudouridine) were found for the leukemia and breast cancer patients, and to a lesser extent for the miscellaneous and brain tumor group. It is concluded that serum nucleoside levels can be determined by radioimmunoassay, and are higher in cancer patients than in controls.

1138 THE SURFACE GLYCOPROTEINS OF A MOUSE MELANOMA GROWING IN CULTURE AND AS A SOLID TUMOR *IN VIVO*. (Eng.) Warren, L. (Univ. of Pennsylvania Sch. of Medicine, Philadelphia, Pa. 19174); Zeidman, I.; Buck, C. A. *Cancer Res.* 35(8):2186-2190; 1975.

The fact that solid melanotic tumors (B16) growing in various mouse organs exhibit glycopeptide patterns similar to those of malignant cells in tissue culture is elucidated. B16 melanotic tumors in various organs of mice were labeled with either [^{14}C]- or [^{3}H]L-fucose or D-glucosamine. Glycopeptides derived from the membrane glycoproteins of these tumors were compared with those of normal tissues by double-label elution patterns from Sephadex G-50 columns. A marked increase of sialic acid-rich, fucose containing glycoeptides (Peak A) was found in the glycoproteins of the surface and internal membranes of melanotic cells. The glycopeptides could be reduced in size by treatment with neuaminidase. Comparison of the glycopeptide patterns of melanoma cells grown in culture and in mice revealed a greater complexity in *in vivo* material. Virtually all of the glycopeptides from melanoma cells grown in culture were of the larger type (Peak A) that correlates well with the malignant state. Comparison of two lines of B16 melanoma cells with greatly differing abilities to form tumors

in lung revealed no significant, reproducible differences in their glycopeptide patterns.

1139 MEMBRANE GLYCOPROTEIN DIFFERENCES BETWEEN NORMAL LACTATING MAMMARY TISSUE AND THE R3230 AC MAMMARY TUMOR. (Eng.) Shin, B. C. (Dep. Biochem., Oklahoma State Univ., Stillwater); Ebner, K. E.; Hudson, B. G.; Carraway, K. L. *Cancer Res.* 35(5):1135-1140; 1975.

Variations in cell surface components between a normal lactating mammary gland and a minimal deviation adenocarcinoma R3230 AC are reported. Membrane fractions were assayed for enrichment in 5'-nucleotidase and Na^{+}-K^{+}-ATPase as a means of evaluating membrane purification. Over a series of seven preparations (four normal and three tumor) the 5'-nucleotidase-specific activities varied from 1.8-4.1 μmoles/hr/mg protein for normal homogenates and from 1.3-3.3 μmoles/hr/mg from tumor homogenates. The activity was localized with the plasma membrane marker on sucrose density gradient centrifugation showing a 15- to 25-fold enrichment in the lightest fraction (F-1). Electrophoretic profiles of polypeptides from membranes of tumor and normal cells showed only relatively minor differences. Glycoprotein patterns were observed by periodate-Schiff staining of acrylamide gels. Two major glycoproteins (GP-II and GP-III) were observed in the F-1 fraction of normal tissue; GP-I was found in the heaviest fraction. A glycoprotein corresponding to GP-II was found in all three sucrose gradients of tumor cells. GP-III was reduced with a new peak of lower molecular weight evident in the lightest fraction. There was also a difference in the amount and position of GP-I in the tumor fractions. There was evidence of galactosyl transferase in tumor membranes but this was not reflected in an increased amount of Golgi bodies. The concentration of glycoprotein in the lightest fraction indicates the presence of cell surface material. These results reinforce the concept of the importance of glycoprotein in neoplastic behavior.

1140 CHANGES IN POLYAMINE METABOLISM IN WI38 CELLS STIMULATED TO PROLIFERATE. (Eng.) Heby, O. (Baltimore Cancer Res. Cent., Md.); Marton, L. J.; Zardi, L.; Russell, D. H.; Baserga, R. *Exp. Cell Res.* 90(1):8-14; 1975.

Quiescent confluent monolayers of WI38 human diploid fibroblasts were stimulated to proliferate by replacement of the exhausted medium with medium containing 10% fetal calf serum; and the cellular content of polyamines, purescine, spermidine, and spermine was studied at various intervals. The putrescine content increased during the pre-replicative phase of the cell cycle, whereas the content of spermidine and spermine did not increase until after the initiation of DNA synthesis. By varying the composition of the stimulating medium, it was possible to alter the percentage of cells that were stimulated to proliferate. Measurement of the cellular polyamine content and ^{3}H-thymidine incorporation into DNA at the time of the maximal rate of DNA synthesis showed that the magnitude of the putrescine accumulation depended on the percentage of cells that were stimulated to proliferate. These results indicate that there may be a connection between polyamine synthesis and subsequent DNA replication.

1141 NUCLEOTIDE COMPOSITION ANALYSIS OF tRNA FROM LEUKEMIA PATIENT CELL SAMPLES AND HUMAN CELL LINES. (Eng.) Agris, P. F. (Div. Biological Sciences, Univ. Missouri, Columbia, Mo. 65201). *Nucleic Acids Res.* 2(7):1083-1091; 1975.

A technique developed for analysis of less than μg quantities of tRNA was applied to the study of human leukemia. Leukocytes from peripheral blood and bone marrow samples of six untreated leukemia patients and cells of five different established human cell lines were maintained for 18 hr in media containing [^{32}P]-phosphate. Incorporation of radioactive phosphate into the cells from the patient samples was slightly less that that of the cell lines. Likewise, incorporation of [^{32}P]-phosphate into the tRNA of the patient samples (approximately 5×10^{6} DPM/μg of tRNA) was also less than that incoporated into the tRNA of the cell lines. The major and minor nucleotide compositions of the unfractionated tRNA preparations from each patient sample and each cell line were determined and compared. Similarities and differences in the major and minor nucleotide compositions of the tRNA preparations are discussed with reference to types of leukemia and the importance of patient sample analysis *vs* analysis of cultured human cells.

1142 BIOCHEMICAL CHARACTERIZATION OF PUTATIVE SUBVIRAL PARTICULATES FROM HUMAN MALIGNANT BREAST TUMORS. (Eng.) Michalides, R. (Meloy Lab., Inc., Springfield, Va.); Spiegelman, S.; Schlom, J. *Cancer Res.* 35(4):1003-1008; 1975.

Putative subviral particulates were isolated from murine and human mammary adenocarcinomas. Minced, homogenized murine mammary tumor obtained from Paris RIII mice, lactating mammary gland from normal NIH Swiss mice, and human malignant and benign breast tumor tissues were subjected to velocity and sucrose equilibrium gradient centrifugation. Material banding at 1.15-1.20 g/ml was treated with Sterox-SL followed by another equilibrium density gradient centrifugation and was assayed by the simultaneous detection test. Particulates isolated from the murine and human adenocarcinomas had the biochemical properties of cores and/or ribonucleoproteins of RNA tumor viruses, i.e., a density of 1.26 g/ml or more in sucrose and RNA-directed DNA polymerase activity associated with a 60-70 Svedberg unit RNA. No such particulates occurred in normal lactating mammary tissue controls or in human benign tumor extracts. That the simultaneous detection of 60-70 Svedberg unit RNA and reverse transcriptase is improved by treatment with Sterox-SL may be due to the elimination of contaminating cell debris which bands at 1.15-1.20 g/ml. These data add further information relevant to characterizing the particles previously identified in human breast cancers.

1143 PHYSICOCHEMICAL CHARACTERIZATION OF NOVIKOFF HEPATOMA MITOCHONDRIAL DNA. (Eng.) White, M. T. (Dep. Mol. Biol. Biochem., Univ. California, Irvine); Wagner, E. K.; Tewari, K. K. *Cancer Res.* 35(4):873-879; 1975.

Mitochondrial DNAs (mtDNA), isolated from rat liver and from Novikoff hepatoma grown as both solid tumor and cells in monolayer culture, were examined by a variety of physicochemical techniques. Purified mtDNA was obtained from DNase-treated, lysed mitochondria centrifuged in preparative CsCl:ethidium bromide density gradients. Liver, tumor and cultured hepatoma cells had identical buoyant densities in isopycnic CsCl equilibrium gradients, and thermal denaturation profiles revealed no significant differences in base composition. Sedimentation analysis in both neutral (2.85 M, pH 8.0) and alkaline (2.85 M, pH 12.3) CsCl at 20 C revealed no significant size differences in mtDNAs from normal and neoplastic cells. Contour length measurements of mtDNA spread on a hypophase cospread with ϕX174 RF11 DNA as an internal size standard were also performed. The neoplastic mtDNAs were more heterogeneous in size. The calculated molecular weights of liver, tumor, and hepatoma cell mtDNAs averaged 10.14 x 10^6, 9.96 x 10^6, and 9.99 x 10^6 daltons, resp. Electron microscopy revealed 4%, 15%, and 18% of the liver, hepatoma cell, and tumor mtDNA, resp., to be in the form of catenated dimers and oligomers. There were 6, 25, and 32 molecules per mitochondrion in liver, tumor and hepatoma cells, resp., as calculated from the results of phenylamine assay. This apparent four- to five-fold increase in neoplastic mtDNA was supported by the finding that there was no difference in the number of mitochondria per mg protein in the three cell types. Significant changes are evident in the Novikoff hepatoma genome. The increase in the number of mtDNA molecules per mitochondrion and the higher proportion of catenated oligometric forms may be due to defects in mtDNA replication and/or the nature of mtDNA attachment to the mitochondrial membranes. The slightly lower molecular weight of both neoplastic cell types may indicate deletions of portions of the mitochondrial genome.

1144 PLEIOTROPIC EFFECTS OF A DNA ADENINE METHYLATION MUTATION (*dam-3*) IN *ESCHERICHIA COLI* K12. (Eng.) Marinus, M. G. (Coll. Med. Dent. New Jersey, Piscataway); Morris, N. R. *Mutat. Res.* 28(1):15-26; 1975.

Comparative studies of the phenotypic traits of *dam+* and *dam-3* (a DNA adenine methylation mutation) *Escherichia coli* K12 strains are reported. The *dam-3* mutation results in a five-fold reduction in the number of 6-methyl-adenine (6-meA) residues in the DNA of *E. coli* or phage λ. The DNA of phage fd appears to be devoid of 6-meA when propagated on *dam-3* bacteria. The phenotypic differences between *dam-3* and *dam+* bacteria include increased free phage in lysogenic *dam-3* cultures; increased sensitivity of *dam-3* to UV irradiation, mitomycin C, and methyl methanesulfonate (MMS); inviability of *dam-3 lex-I* strains; low molecular weight of DNA in *dam-3* bacteria in the absence of DNA ligase; and increased rate of DNA degradation in *dam-3 recA* strains. These facts indicate that a function of 6-meA is to protect DNA from an endonuclease. It is suggested that the sensitivity of *dam-3* bacteria to UV irradiation, mitomycin C, and MMS is a secondary consequence due to saturation of repair enzymes rather than a primary consequence of adenine undermethylation and that the *recA* gene product functions to stabilize breaks in DNA and prevent degradation by extraneous nucleases

1145 THE GLYCOSAMINOGLYCANS IN HUMAN HEPATIC HEPATIC CANCER. (Eng.) Kojima, J. (Cent. Adult Dis., Osaka, Japan); Nakamura, N.; Kanatani, M.; Ohmori, K. *Cancer Res.* 35(3):542-547; 1975.

A method is proposed for the measurement of glycosaminoglycans isolated from human liver. The method combines cellulose acetate electrophoresis and enzymatic digestion with mucopolysaccharidases. Twenty hepatic tumors were compared with seven healthy control livers and three cirrhotic livers. After centrifugation, protein digestion, and dialysis, the uronic acid content was measured in both sulfated and non-sulfated glycosaminoglycans. Chondroitin sulfate and hyaluronic acid composition exceeded 90%. Addition of distilled water allowed enzymatic analysis of the glycosaminoglycans with various mucopolysaccharides (*Streptomyces* hyaluronidase, testicular hyaluronidase and chondroitin-ABC). The components of glycosaminoglycans were as follows: hyaluronic acid, chondroitin, dermatan sulfate, heparin and/or heparan sulfate. Electrophoretic separation was carried out to confirm complete digestion with each enzyme. The average value of glycosaminoglycans in tumor nodules is 7-fold the value in control livers. Such an increase is also noted in cirrhotic livers. In hepatocellular carcinoma, the amounts of chondroitin and/or chrondroitin sulfate and hyaluronic acid are increased 33 and 10 times, resp., over healthy livers. The dermatan level is higher than normal in both cancerous and cirrhotic livers. Finally, the ratio of dermatan sulfate to heparan sulfate is higher in cirrhosis and carcinomas. The comparative results are significant, yet the mechanism for abnormal accumulation has not been elucidated.

1146 A TRANSFORMATION-DEPENDENT DIFFERENCE IN THE HEPARAN SULFATE ASSOCIATED WITH THE CELL SURFACE. (Eng.) Underhill, C. B. (Dep. Biochem., Univ. Washington, Seattle); Keller, J. M. *Biochem. Biophys. Res. Commun.* 63(2):448-454; 1975.

Glycosaminoglycans from the surface of normal mouse cells (3T3) and from transformed cells (SV40-3T3, 3T6) were compared with a double label technique. The glycosaminoglycans were released by trypsin digestion and separated by ion-exchange chromatography into hyaluronic acid, heparan sulfate, and chondroitin sulfate. No difference was apparent in either the hyaluronic acid or chondroitin sulfate fractions; however, the heparan sulfate from 3T3 cells eluted from DEAE cellulose at a higher ionic strength than that from transformed cells. This altered behavior implies a structural difference in the cell surface heparan sulfate which appears to be dependent on transformation.

1147 FIBRINOLYTIC ACTIVITY IN A HUMAN FIBROSARCOMA CELL LINE AND EVIDENCE FOR THE INDUCTION OF PLASMINOGEN ACTIVATOR SECRETION DURING TUMOR FORMATION. (Eng.) Jones, P. A. (Univ. Southern California Sch. Medicine, Los Angeles, Calif. 90027); Laug, W. E.; Benedict, W. F. *Cell* 6(2):245-252; 1975.

Seven clones were isolated from the HT1080 fibrosarcoma cell line in an adult man using a fibrinagarose overlay technique. Three of these clones induced lysis of the fibrin overlay, whereas four did not. The extracellular and intracellular levels of protease were then measured using ^{125}I-fibrin plates incubated with acid-treated human serum. The extracellular protease can be directly assayed in the medium from cells incubated with 10% fetal calf serum. Although there were large differences in the amounts of protease secreted by these two sets of clones, the intracellular levels of protease were similar. No significant differences were found between the abilities of the cells to grow in soft agar or as tumors in immunosuppressed hamsters. However, cells grown from tumors, derived from all the low secretors of protease, showed an increase in the amount of protease secreted. It appeared, therefore, that the secretion of protease might be selected for or induced during tumor growth. Further detailed studies with one of the low secreting clones (clone E) suggested an inductive rather than a selective mechanism for this increase in extracellular plasminogen activator.

1148 EFFECT OF PROTEASES ON ACTIVATION OF RESTING CHICK EMBRYO FIBROBLASTS AND ON CELL SURFACE PROTEINS. (Eng.) Blumberg, P. M. (Harvard Medical Sch., Boston, Mass. 02115); Robbins, P. W. *Cell* 6(2):137-147; 1975.

The relationship between activation of resting chick embryo fibroblasts by proteases and proteolytic alteration of the cell surface has been investigated. Five different proteases were examined: trypsin, collagenase, plasmin, α-chymotrypsin, and thrombin. All of these proteases, when added to the culture medium at concentrations of 0.08-2.2 μg/ml, stimulated deoxyglucose uptake and induced cell division. The absolute levels of stimulation depended on the specific protease. Activation ranged from a doubling in cell number in 24 hr for trypsin and thrombin down to a 47% increase in cell number for α-chymotrypsin. Except in the case of thrombin, the stimulatory effects of these proteases correlated with breakdown of Z, the major chick surface protein. Z is revealed by lactoperoxidase-catalyzed iodination and disappears upon transformation. In the case of thrombin, stimulatory concentrations brought about no detectable loss of surface components. Thus loss of Z is not a necessary condition for activation of chick fibroblasts; it may be a sufficient condition for activation of part of the cell population.

1149 POLY(A) POLYMERASE OF BOVINE LYMPHOSARCOMA. (Eng.) Keshgegian, A. A. (Sch. Med., Univ. Pennsylvania, Philadelphia); Meltzer, S. M.; Furth, J. J. *Cancer Res.* 35(5):1141-1146; 1975.

The ability of isolated poly(A) polymerase to use various polynucleotides as a primer and the properties and primer preference of the enzymes from lymphosarcoma were studied. Poly(A) polymerase was extensively purified from low-salt extracts of bovine lymphosarcoma. The enzyme was found to be Mn^{2+} dependent, to require an oligonucleotide or RNA primer, to incorporate only ATP, and to be inhibited by other ribonucleotides or deoxynucleotides. Oligoadenylate and ribosomal RNA were demonstrated to be good primers for the enzyme; transfer RNA and poly(A) were poor. RNA transcribed *in vitro* by homologous RNA polymerase was an efficient primer. The properties of the enzyme were found to be similar to the properties of the Mn^{2+}-activated poly(A) polymerase of calf thymus. Approximately the same amount of enzyme was present in lymphosarcoma and calf thymus. The demonstration that RNA synthesized *in vitro* by mammalian RNA polymerase is an efficient primer for poly(A) polymerase provides evidence for the role of poly(A) polymerase in the metabolism of DNA-like RNA *in vivo* and suggests that the synthesis and processing of RNA can be reproduced *in vitro* by the coupling of the two enzymes.

1150 PURIFICATION AND PROPERTIES OF NUCLEAR AND CYTOPLASMIC DNA POLYMERASES FROM JLS-V9 CELLS. (Eng.) Bandyopadhyay, A. K. (Frederick Cancer Res. Cent., Md.). *Arch. Biochem. Biophys.* 166(1):72-82; 1975.

The partial purification and properties of four DNA polymerases, two from the nucleus and two from the cytoplasm, are described. Cells from an established mouse bone marrow cell line (JLS-V9) were grown in suspension culture and harvested in log phase at 4 to 6 x 10^5 cells/ml. The yield of cells was about 1 g wet weight/3 x 10^9 cells. Four to five g of wet cells were suspended in 20 ml 0.01 M Tris·HCl buffer containing 0.002 M dithiothreitol and 15% glycerol. Ten ml aliquots of cells were homogenized, giving breakage of more than 99%. Total volume of the homogenate was 25 ml. Solid sucrose was added to the homogenate to bring the concentration of sucrose to 0.35 M. The homogenate was centrifuged and the supernatant used for the purification of the cytoplasmic enzyme. The pellet was resuspended and further centrifuged. The pellet was again resuspended and the suspended nuclei were centrifuged at 1200 *g* for 10 min. The supernate was discarded and the procedure was repeated once. The resulting purified intact nuclei were used for the purification of nuclear enzyme. The cytoplasmic enzymes were purified by sequential column chromatography in the following steps: DEAE cellulose, phosphocellulose, glycerol gradient centrifugation, and DNA-cellulose, to yield two enzymes. The first was purified 2100 times and was designated cytoplasmic polymerase I ($PolyC_1$) and the second was purified 5000 times and designated cytoplasmic polymerase II ($PolyC_2$). The nuclear enzymes were also purified by sequential column chromatography (DEAE cellulose, ammonium sulfate precipitation, phosphocellulose column, DNA-cellulose column and RNA-cellulose column) to yield two enzymes. The first was purified 7000 times and was designated as nuc-

lear polymerase I ($PolyN_1$) and the second was purified 3700 times and designated as nuclear polymerase II ($PolyN_2$). For further purification several procedures such as Sephadex G-200 gel filtration, hydroxylapatite column chromatography, and density gradient centrifugation, were applied with unsuccessful results. Each of the polymerases required all the deoxynucleoside-5'-triphosphates to synthesize DNA, using activated DNA as a primer-template, and could copy the ribonucleotide strand of hybrid templates, but their rate of efficiency varied. The molecular weights of these DNA polymerases ranged from 35,000-160,000. Three of four DNA polymerases are probably a single polypeptide chain, since they have a single major band in polyacrylamide gel electrophoresis and one enzymatically active peak in guanidine hydrochloride gel filtration. The results suggest that $PolyN_2$ and $PolyC_2$ are the same enzyme and that $PolyN_1$ and $PolyC_1$ are distinct enzymes.

1151 INCREASES IN THE ACTIVITY OF THE SOLUBILIZED RAT LIVER NUCLEAR RNA POLYMERASES FOLLOWING PARTIAL HEPATECTOMY. (Eng.) Organtini, J. E. (Temple Univ. Sch. Medicine, Philadelphia, Pa. 19140); Joseph, C. R.; Farber*, J. L. *Arch. Biochem. Biophys.* 170(2):485-491; 1975.

Nuclei isolated 18 hr following partial hepatectomy in the female Wistar rat synthesized RNA *in vitro* at twice the rate of liver nuclei from sham-operated animals. This increased RNA synthesis was accompanied by an increase in the α-amanitin-insensitive proportion of the total activity. Twice as much RNA polymerase activity was solubilized from nuclei from hepatectomized animals as from sham-operated animals. Fractionation of this solubilized RNA polymerase activity on DEAE-Sephadex showed increases in the activity of both polymerase I and polymerase II, with a proportionally greater increase in polymerase I. Such an increase in RNA polymerase activity independent of the endogenous chromatin template was also demonstrated by inhibiting the function of the chromatin template in whole nuclei with actinomycin D and then assaying the polymerase activity with poly[d(A-T)]. It is concluded that increases in the activity of the RNA polymerases themselves can account entirely for the changes in the rates of RNA synthesis 18 hr following partial hepatectomy.

1152 CHANGES IN LYMPHOID CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE METABOLISM DURING MURINE LEUKEMOGENESIS. (Eng.) Kemp, R. G. (Medical Coll. Wisconsin, Milwaukee, Wis. 53233): Hsu, P.-Y.; Duquesnoy, R. J. *Cancer Res.* 35(9): 2440-2445; 1975.

Cyclic adenosine 3':5'-monophosphate (cyclic AMP) levels were investigated in preleukemic AKR mouse thymus cells. These levels were slightly increased compared with nonleukemic thymus cells, but were markedly reduced in leukemic cells. Adenylate cyclase activity rose during the preleukemic and leukemic phases of leukemogenesis. Although the drop of epinephrine-induced stimulation of thymus adenylate cyclase in the preleukemic phase was probably age-related, there was an additional decrease of adenylate cyclase activation by epinephrine in leukemic cells. Cyclic AMP phosphodiesterase activity was slightly higher in preleukemic cells and more than four-fold higher in leukemic thymus, compared with nonleukemic AKR thymus. It is suggested that cyclic AMP phosphodiesterase is largely responsible for the low levels of cyclic AMP in leukemic cells. Significant changes in cyclic AMP metabolism are already detectable before neoplastic cells may be found in the thymus.

1153 CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE LEVELS AND ACTIVITIES OF RELATED ENZYMES IN NORMAL AND LEUKEMIC LYMPHOCYTES. (Eng.) Monahan, T. M. (Univ. Texas Medical Branch, Galveston, Tex. 77550); Marchand, N. W.; Fritz, R. R.; Abell, C. W. *Cancer Res.* 35(9):2540-2547; 1975.

The role of cyclic adenosine 3':5'-monophosphate (cyclic 3':5'-AMP) in the regulation of cell division in lymphocytes obtained from healthy donors and from patients with chronic lymphocytic leukemia (CLL) was investigated by determining the levels of cyclic 3':5'-AMP and glycogen and also the activities of several enzymes that are closely associated with the metabolism of these cellular components. Intracellular levels of cyclic 3':5'-AMP were measured in normal and CLL lymphocytes in nondividing, dividing, and quiescent [after phytohemagglutinin (PHA) addition] states. In normal lymphocytes, the levels of cyclic 3':5'-AMP fluctuated throughout the cell cycle after PHA addition, whereas in CLL lymphocytes the levels were approximately three-fold lower than in normal cells and remained relatively constant before, during, and after mitogenic stimulation. Normal cells contained approximately three-fold lower levels of glycogen than CLL cells, whereas glycogen phosphorylase activities were increased two- to four-fold above those in nondividing cells in normal but not in CLL lymphocytes after stimulation with PHA. Furthermore, cyclic 3':5'-AMP phosphodiesterase activities were higher in CLL lymphocytes than in normal ones. It was demonstrated that (a) the intracellular levels of cyclic 3':5'-AMP differ in these two cell types; (b) the levels of cyclic 3':5'-AMP and glycogen qualitatively correlate with the activities of the enzymes that are related to these components; and (c) an inverse relationship between the levels of cyclic 3':5'-AMP and cell growth exists in mitogen-stimulated lymphocytes from healthy donors but not from patients with CLL. These biochemical differences are presumed to exist between normal and "leukemic" lymphocytes, but alternatively they may reflect normal populations of immunologically distinct lymphocytes.

1154 CHARACTERISATION OF CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE PHOSPHODIESTERASE FROM WALKER CARCIMONA SENSITIVE AND RESISTANT TO BIFUNCTIONAL ALKYLATING AGENTS. (Eng.) Tisdale, M. J. (St. Thomas's Hosp. Med. Sch., London, England). *Biochim. Biophys. Acta* 397(1):134-143; 1975.

Walker carcinoma cell lines sensitive or resistant to bifunctional alkylating agents were found to contain multiple forms of cyclic AMP phosphodiesterase (3':5'-

cyclic AMP 5'-nucleotidohydrolase, EC 3.1.4.17). These activities were resolved using Sepharose 6B gel filtration. The enzyme appeared to occur in four active forms of apparent molecular wt of >1,000,000, 430,000, 350,000, and 225,000, when assayed at low substrate concentrations. While the ionic strength of the buffer affected the predominance of the different forms, the presence of cyclic AMP at 10^{-6} M had no effect on aggregation or dissociation of the enzyme. An activity shift from high moleculat wt forms of the enzyme to low molecular wt forms was found in the resistant tumor at low substrate concentration. No change in elution profile between sensitive and resistant tumors was observed for the low affinity form of the enzyme. The pH optima of the enzymes with both high and low affinity for the substrate was found to be pH 8.0 in the sensitive line. In the resistant tumor the pH optima of the high affinity form is shifted to pH 8.4 while the low affinity form remains at pH 8.0. The high affinity forms of the phosphodiesterase in the sensitive and resistant tumor also differed in their inhibition by theophylline. In both cases, inhibition was of the competitive type with K_i values for the sensitive and resistant lines being 2.35 and 0.32 mM, resp. There was no significant difference in the inhibition of the low affinity form between the sensitive and resistant tumor. It is suggested that this tissue specificity of phosphodiesterase may provide an important new target for anti-tumor chemotherapy, by administration of the agents which specifically affect the phosphodiesterase of the neoplastic tissue.

1155 URIDINE-CYTIDINE KINASE: PURIFICATION FROM A MURINE NEOPLASM AND CHARACTERIZATION OF THE ENZYME. (Eng.) Liacouras, A. S. (Natl. Cancer Inst., Bethesda, Md.); Anderson, E. P. *Arch. Biochem. Biophys.* 168(1):66-73; 1975.

Uridine-cytidine kinase, which catalyzes the phosphorylation of uridine or cytidine to the respective nucleoside monophosphate, was purified 250-fold from extracts of acetone powders from the murine mast cell tumor P815. The purification procedure involved chromatography on DEAE-cellulose, ammonium sulfate fractionation, and separation on hydroxyapatite. When assayed at 37 C, the activity of the enzyme for the uridine substrated was twice that for cytidine, and this ratio remained essentially constant throughout the purification. The requirement of this reaction for a divalent cation could best be met by Mg or Mn, but several other metals could partially substitute. The enzyme had a broad pH optimum around 6.5-7.0. The purified enzyme had a broad specificity for phpsphate donor, by ATP and dATP were the most effective. With ATP as a donor, uridine, cytidine, 5-fluoridine, 6-azauridine, and 5-azacytidine were phosphorylated by the enzyme, but thymine, riboside, deoxyribosides, and cytosine arabinoside did not serve as substrates. It is suggested that further investigations on the role of the various nucleoside triphosphates in this reaction are necessary.

1156 URIDINE-CYTIDINE KINASE: KINETIC STUDIES AND REACTION MECHANISM. (Eng.) Liacouras, A. S. (Natl. Cancer Inst., Bethesda, Md.); Garvey, T. Q., III; Millar, F. K.; Anderson, E. P. *Arch. Biochem. Biophys.* 168(1):74-80; 1975.

The initial velocity pattern was determined for uridine-cytidine kinase purified from the murine mast cell neoplasm P815. With either uridine or cytidine as phosphate accepter, and ATP as phosphate donor, the pattern observed was one of intersecting lines, ruling out a ping-pong reaction mechanism, and suggesting that the reaction probably proceeds by the sequential addition of both substrates to the enzyme to form a ternary complex, followed by the sequential release of the two products. This pattern was obtained whether the reaction was run in 0.01 M potassium phosphate buffer, pH 7.5, or in 0.1 M Tris-HCl, pH 7.2. When analyzed by the Sequen computer program, the data indicated an apparent K_m of the enzyme for uridine of 1.5×10^{-4} M, an apparent K_m for cytidine of 4.5×10^{-5} M, and a K_m for ATP, with uridine or cytidine as phosphate acceptor, of 3.6×10^{-3} M or 2.1×10^{-3} M, resp. The V was 1.83 μM phosphorylated /min/mg of enzyme protein for the uridine kinase reaction and 0.91 μM for the cytidine kinase reaction. It is suggested that the difference in K_m for the two nucleosides may mean that the intracellular pool of cytidine available to the enzyme is considerably smaller than the pool of uridine.

1157 PEPTIDYL PROLINE-HYDROXYLASE IN ADULT, DEVELOPING, AND NEOPLASTIC RAT TISSUE. (Eng.) Zimmerberg, J. (Harvard Med. Sch., Boston, Mass.); Greengard, O.; Knox, W. E. *Cancer Res.* 35(4):1009-1014; 1975.

The peptidyl proline hydroxylase (PPH) activities in adult, fetal, and neoplastic rat tissues were determined by a sensitive assay system optimally supplemented with tritiated protocollagen substrate and cofactors. The substrate was prepared according to a previous method except that preincubating chick bones prior to (^{3}H)proline treatment was avoided. Whole homogenates and both the supernatant and particulate fractions of centrifuged tissue homogenates were assayed. Less than 50% of the total PPH activity of all tissues except adult kidney and renal carcinoma MK1 was soluble; the activity of the particulate portion (concentrated in the mitochondrial and microsomal fractions) was doubled by pretreatment with 0.5% Triton X-100. Among normal adult tissues, lung had the highest total PPH activity (2.4 times that of liver) and small intestine had the lowest (25% of liver). Lactating mammary gland, kidney, skin, brain, and muscle had activities 66%, 64%, 62%, 59% and 52%, resp., of that of liver. Fetal tissues at 19 days contained 3-8 times more PPH than the corresponding adult tissues, and less than 21% of the total activity was usually soluble. In lung, liver, kidney and brain, the total PPH declined rapidly during the last two days of gestation. Neonatal brain and lung had activities similar to the analogous adult tissues while liver and kidney were about twice as high. Administration of 0.05 mg cortisol to fetal rats resulted in enhancement of the decline in level of PPH in lung, liver, and skull, which occurred during normal development of the rats. In renal, mammary, muscle and hepatic tumors, PPH activities were 4-10 times higher than in the cognate adult tissues; even in well-differentiated,

slow growing tumors, the activity was higher than in normal, mature or immature tissue with the exception of fetal skull and lung. PPH activity is thus a sensitive indicator of neoplastic growth.

1158 GLUTAMINE-DEPENDENT ASPARAGINE SYNTHETASE IN FETAL, ADULT AND NEOPLASTIC RAT TISSUES. (Eng.) Huang, Y.-Z. (Harvard Med. Sch., Boston, Mass.); Knox, W. E. *Enzyme* 19(5/6):314-328; 1975.

Three enzyme reactions related to asparagine synthesis were studied in inbred Kx rat tissues: formation of aspartylhydroxamate, either from aspartate or by transfer from asparagine, and actual synthesis of asparagine from aspartate. Actual asparagine synthesis occurred at one-thousandth the rate of the other two reactions. Detection of asparagine synthetase activity involved separating ^{14}C-asparagine from the substrate, ^{14}C-aspartate, by elution of ^{14}C-asparagine from an acid alumina column in the presence of 30 mM carrier L-asparagine. Optimal conditions for quantitative assay of asparagine synthesis were determined in fetal liver extract. Demonstrable activity in liver fell six days after birth to 20% of the fetal value and decreased thereafter to the low adult value. Adult pancreas was the most active tissue found. Spleen and small intestine had the highest activities among other adult tissues. Generally, tumors had more activity than most normal adult tissues. Activities were higher in tumors with faster growth rates. The asparagine synthetase of fetal liver extracts was significantly inhibited when combined with adult liver or tumor extracts. The inhibitor is nondializable, heat labile, and precipitated in the same ammonium sulfate as the synthetase. It is suggested that the inhibitor observed *in vitro* also occurs *in vivo* and represents a physiological regulatory mechanism. It is concluded that the measured activities in tissues are not demonstrably parallel to the concentrations of asparagine synthetase in the tissues.

1159 MORPHOLOGICAL ALTERATIONS AND GANGLIOSIDE SIALYLTRANSFERASE ACTIVITY INDUCED BY SMALL FATTY ACIDS IN HELA CELLS. (Eng.) Simmons, J. L. (Natl. Inst. Neurol. Dis. Stroke, Bethesda, Md.); Fishman, P. H.; Freese, E.; Brady, R. O. *J. Cell Biol.* 66(2):414-424; 1975.

The specificity and kinetics of sialytransferase induction are reported. Incubation of HeLa cells in the presence of mM concentrations of propionate, butyrate, or pentanoate increased the specific activity of CMP-sialic acid:lactosylceramide sialytransferase 7-20-fold within 24 hr. Longer-chain saturated fatty acids or acetate were much less effective; decanoate showed no induction. Unsaturated fatty acid analogs of butyrate and other compounds were ineffective. Only the three most effective compounds also produced characteristic smooth extended cell processes. Butyrate (5 mM) induced the sialyltransferase after a four hr lag, producing maximum specific activity by 24 hr. The amount of sialyl-lactosylceramide, the glycolipid product of the enzyme, increased during that time 3.5 times more than in control cultures. No other glycosphingolipid enzyme was significantly altered by butyrate exposure. The cellular shape changes occurred two to three hr later than the increase of sialyltransferase activity, and both processes required the continuous presence of inducer and the synthesis of RNA and protein but not the synthesis of DNA or the presence of serum. It is suggested that the modulation of sialyltransferase activity may provide a convenient model to explore the role of these complex carbohydrates in malignant processes.

1160 ACETYL-COENZYME A: 1,4-DIAMINOBUTANE *N*-ACETYLTRANSFERASE: ACTIVITY IN RAT BRAIN DURING DEVELOPMENT, IN EXPERIMENTAL BRAIN TUMOURS AND IN BRAINS OF FISH OF DIFFERENT METABOLIC ACTIVITY. (Eng.) Seiler, N. (Max-Planck-Institut fur Hirnforschung, Arbeitsgruppe Neurochemie, Frankfurt/M West Germany); Lamberty, U.; Al-Therib, M. J. *J. Neurochem.* 24(4):797-800; 1975.

The influence of different physiological states on the activity of the putrescine acetylating enzyme 1,4 diaminobutane *N*-acetyltransferase (putrescine acetylase) was studied in Wistar rats and in trout. Putrescine acetylase activity in the brains of one-day-old animals was three times higher than in adults (360 days). Cell nuclei of adult rats showed higher specific activities of the enzyme than did microsomes while the opposite was evident in immature brains. Nitro-ethylurea-induced gliomas contained both enhanced enzyme levels and acetylase activity. The difference in activity between tumor and non-tumor tissue was the same as the difference between microsomal and nuclear activity in immature brains. Spermidine and spermine concentrations were not altered in tumor-bearing animals. Increased putrescine acetylase activity was found only in those brains in which tumor could be observed macroscopically. In trout progressively adapted to higher environmental temperatures (5 C to 23 C) there was a linear increase in acetylase activity of 0.1 nmole/mg protein whereas the brain concentration decreased concomitantly by 0.3 nmole/mg protein. The decrease in enzyme activity in the brain microsomal and nuclei fractions with aging indicates that the enzyme-bound structural elements decrease with differentiation of the nerve cells.

1161 ADENYLATE AND GUANYLATE CYCLASE ACTIVITIES AND CELLULAR DIFFERENTIATION IN RAT SMALL INTESTINE. (Eng.) Quill, H. (Massachusetts General Hosp., Boston, Mass. 02114); Weiser*, M. M. *Gastroenterology* 69(2):470-478; 1975.

Adenylate and guanylate cyclase activities were measured in rat small intestinal villus and crypt cells to determine possible correlations with cellular differentiation. Isolated intestinal cells were prepared by a method which effectively separates differentiated villus cells from undifferentiated crypt cells. Crypt cells had a significantly lower guanylate cyclase activity than villus cells. Adenylate cyclase activity was higher in crypt cells than villus cells, although the difference was less striking than the reverse gra-

dient observed for guanylate cyclase. There was no gradient of activity for cyclic guanosine 3':5'-monophosphate phosphodiesterase. However, cyclic adenosine 3':5'-monophosphate phosphodiesterase activity was lower in villus cells. No villus to crypt gradient of cyclic adenosine 3':5'-monophosphate concentration was detected in mucosa frozen rapidly in liquid nitrogen. The properties and subcellular localization of the cyclases were also evaluated, and of particular interest was the localization of guanylate cyclase to the microvillus membrane and the confirmation of adenylate cyclase activity in the lateral-basal membrane. The villus to crypt gradient of guanylate cyclase suggests that this enzyme has a specialized role in the differentiated villus cell. The contrasting subcellular localization of the cyclases suggests that the cyclases may be interrelated, possibly reflecting the epithelial cell polarity for absorption and secretion.

1162 AMINOPEPTIDASES AND ARYLAMIDASES IN NORMAL AND CANCER TISSUES IN HUMANS. (Eng.) Tamura, Y. (Sch. Med., Tokushima Univ., Japan); Niinobe, M.; Arima, T.; Okuda, H.; Fujii, S. *Cancer Res.* 35 (4):1030-1034; 1975.

Aminopeptidases and arylamidases were solubilized from sonically extracted suspensions of tissue from normal human liver, stomach, lung, ileum, colon, rectum and kidney and of tissue from human hepatocellular carcinoma, stomach adenocarcinoma, and squamous cell carcinoma of the lung by treatment with bromelain (100 μg/mg). Multiforms of the enzymes were then separated by triethylaminoethyl cellulose column chromatography. When the activities of the multiforms were assayed by direct colorimetric methods, they showed different substrate specificities toward L-leucyl-β-naphthylamide and L-amino acid amides. Although normal and cancerous liver tissue both gave three chromatographic peaks, the substrate specificities of the corresponding peaks were different. The activity of aminopeptidase toward L-leucyl-β-naphthylamide was lower in hepatic cancer tissue than in normal liver. The activity in stomach cancer tissue was higher than in normal stomach and gave one more peak than did normal tissue. Lung cancer tissue gave one less peak than normal tissue and had abnormally low aminopeptidase activity. Arylamidase activity in lung cancer tissue was similar to that in normal lung. Aminopeptidase and arylamidase activities were found in all tissues tested and were especially high in kidney, liver, and ileum. The observed differences in aminopeptidase and arylamidase activities of multiforms from different sources may afford a clue to the metabolic disturbances in cancerous tissues and may serve as a diagnostic tool.

1163 GROWTH HORMONE AND PROLACTIN SECRETION IN THE CARCINOID SYNDROME. (Eng.) Feldman, J. M. (Duke Univ. Medical Center, Durham, N. C. 27710); Plonk, J. W.; Bivens, C. H.; Lebovitz, H. E.; Handwerger, S. *Am. J. Med. Sci.* 269(3):333-347; 1975.

Plasma growth hormone (GH) and plasma prolactin (PRL) levels were evaluated in ten patients with metastatic carcinoid tumors and the carcinoid syndrome ("active tumors") and seven patients with metastatic carcinoid tumors without the carcinoid syndrome ("inactive tumors"). The patients with active tumors has elevated serum serotonin levels and increased urinary 5-hydroxyindoleacetic acid (5-HIAA); these values were normal in patients with inactive tumors. Forty-five per cent of patients with active tumors had elevated fasting plasma GH levels that were either not suppressed or showed a paradoxical increase in response to iv glucose. There was a positive correlation between the plasma GH levels and serotonin production by the tumor. Twenty-eight percent of patients with inactive tumors had elevated fasting plasma GH levels. GH levels were decreased by the administration of serotonin antagonists in some but not all of the patients. Parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, caused a paradoxical rise in GH levels. GH release in response to insulin hypoglycemia was normal. Plasma prolactin levels were normal in most of the patients with metastatic carcinoid tumors. PCPA administration did not systematically alter plasma prolactin levels. It is concluded that elevated plasma GH levels are frequently present in patients with the carcinoid syndrome. Both serotonin produced by the tumors and the tumor itself may be responsible for the elevated GH levels.

1164 HORMONE DEPENDENCE AND ESTRADIOL RECEPTORS IN THE D SERIES OF MAMMARY NODULE OUTGROWTH LINES AND TUMORS. (Eng.) Medina, D. (Baylor Coll. Medicine, Houston, Tex. 77025); Iramain, C. A.; Clark, J. H. *Cancer Res.* 35(9):2355-2360; 1975.

Preneoplastic mammary nodule outgrowth lines were examined for their ability to grow and produce tumors in ovariectomized BALB/c mice. In addition, these lines and tumors derived from them were investigated for cytoplasmic estrogen receptor proteins. Early ovariectomy, performed within four wk after transplantation, slightly delayed but did not permanently block the ability of three different nodule lines to completely fill the fat pad with nodule tissue. Ovariectomy performed later than four wk after transplantation or adrenalectomy performed early or late had no effect on nodule growth. Neither early nor late ovariectomy or adrenalectomy had an effect on the maintenance of the nodule alveolar phenotype. Ovariectomy performed three wk after transplantation had little effect on the tumor potential of the low oncogenic line D1 or the high oncogenic line D2. Samples of nodule outgrowths transplanted from ovariectomized mice responded to a chemical carcinogen in a similar manner, as did nodule outgrowths transplanted from the control experiments. Thus, the altered hormonal environment in ovariectomized mice did not select for subpopulations of nodule cells with altered tumorigenic potentials. The ovarian independence of the nodule lines and tumors derived from them was correlated with a very low level of cytoplasmic estrogen receptor. The nature of the independence on ovarian hormonal control for tumorigenisis in these mammary tumor virus-free lines is indicated.

1165 METABOLIC PARAMETERS IN WOMEN WITH METASTATIC BREAST CANCER. (Eng.) Carter, A. C. (Dept. Medicine, State Univ. New York, Brooklyn); Lefkon, B. W.; Farlin, M.; Feldman, E. B. *J. Clin. Endocrinol. Metab.* 40(2):260-264; 1975.

Data of glucose tolerance, hormone secretion, and metabolic response to growth hormone in groups of 6-11 breast cancer patients and healthy women are compared. Baseline metabolic and hormone secretion data acquired from a large number of breast cancer patients are presented. Mean glucose tolerance was impaired in women with breast cancer. Mean plasma glucose at 1 hr was significantly greater than in healthy subjects. Insulin secretion was delayed and prolonged in cancer patients. Mean baseline level of growth hormone in cancer patients (1.8 ± 0.62 ng/ml) was significantly greater than mean growth hormone (0.74 ± 0.23 ng/ml) in healthy subjects. In cancer patients and control subjects given saline, free fatty acids (FFA) increased similarly. After injection of human growth hormone, FFA levels were not significantly changed in cancer patients but were much greater in the controls. Cortisol and protein-bound iodine levels were normal and there was no lipolytic factor in the sera of breast cancer patients. The changes in breast cancer patients were not attributable to age, obesity, inanition, or stress. It is suggested that the incidence of breast cancer may be increased in patients with such metabolic defects, or the tumor may adversely affect the tissue metabolism of the host.

1166 PROLACTIN AND ESTROGEN BINDING IN TRANSPLANTABLE HORMONE-DEPENDENT AND AUTONOMOUS RAT MAMMARY CARCINOMA. (Eng.) Costlow, M. E. (Univ. Texas Health Sci. Cent., San Antonio); Buschow, R. A.; Richert, N. J.; McGuire, W. L. *Cancer Res.* 35(4):970-974; 1975.

Two transplantable rat mammary adenocarcinoma sublines, MA and MD, derived from the same parent tumor (MTW9) were studied with regard to hormone dependence and hormone binding site content. Previous classification of MA growth as autonomous was confirmed when the tumor showed equal growth in intact, ovariectomized, or hypophysectomized rats. The MD tumor has hormone dependent growth because it grew in intact rats but promptly regressed following ovariectomy. Various concentrations of ^{125}I-labeled ovine prolactin were used in prolactin binding studies. With excess unlabeled hormone competitor, total ^{125}I-prolactin binding was diminished by 85% and 75% for the MD and MA tumors, resp. The MA line bound much less ^{125}I-prolactin in the absence of competitor, suggesting fewer hormone binding sites. Using other hormones as competitors, experiments showed the MD tumor binding to be specific in prolactin recognition. These sites are destroyed by trypsin and are thus protein, and prolactin binding is saturable ($Kd \sim 2 \times 10^{-9}$ M) in both tumors. The number of binding sites in the MA tumor as determined by Scatchard analysis was only 1.0 fmole/mg tissue as compared to 4.2 fmoles/mg in the MD tumor. Prolactin binding site DNA content was 1.01 ± 0.58 and 0.15 ± 0.19 fmoles/µg DNA for the MD and MA tumors, resp. Incubation of cytosol fractions of both tumors with 0.015-0.2 pmole [^{3}H]estradiol brought about a mean 8-fold reduction in MA estradiol-binding sites as compared to MD although binding affinity remained unchanged. Nuclei of tumors excised after host rats were injected i.p. with [^{3}H]estradiol (0.1 µCi) showed unimpaired ability in both tumors to translocate estradiol into the nucleus. The reduction in both prolactin and estrogen binding sites in the autonomous MA line may result in an incomplete recognition of the tumor cells as a target for the circulating hormones and an absence of endocrine control over inherent cancer cell growth.

1167 PRODUCTION OF SEZARY-LIKE CELLS FROM NORMAL HUMAN LYMPHOCYTES. (Eng.) Yeckley, J. A. (Univ. Colorado Sch. Med., Denver); Weston, W. L.; Thorne, E. G.; Krueger, G. G. *Arch. Dermatol.* 111(1): 29-32; 1975.

Lymphocyte-rich cell populations were incubated for 72 hr with pokeweed mitogen (0.025 ml) or phytohemagglutinin (0.05 ml), to determine if lymphocytes from healthy humans could be stimulated to assume the morphologic appearance of Sezary cells. Differential cell counts performed by light microscopy on epoxy resin-embedded sections (0.5 µ) showed that 5-11% of the phytohemagglutinin-stimulated lymphocytes and 6-8% of the pokeweed mitogen-stimulated cells had the cerebriform nucleus characteristic of Sezary cells. The morphological results were confirmed by electron microscopy. The major ultrastructural feature of the Sezary-like cells was a lobular serpentine nucleus with clumped peripheral heterochromatin. The cells had little or no rough endoplasmic reticulum, contained no large lysosomes, and lacked prominent Golgi apparatus. Production of Sezary-like cells from stimulated lymphocytes of normal subjects suggests that cells with cerebriform nuclei may represent reactive lymphocytes. Lymphocyte stimulation may explain the presence of Sezary-like cells in benign inflammatory dermatoses as well as in malignant disease.

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1169 SERUM COPPER LEVELS IN PATIENTS WITH SOLID TUMORS. (Eng.) Kolarić, K. (Central Inst. Tumors and Allied Diseases, Zagreb, Yugoslavia); Roguljić, A.; Fuss, V. *Tumori* 61(2):173-177; 1975.

1170 A HUMAN GASTRIC CHORIOCARCINOMA CELL LINE WITH HUMAN CHORIONIC GONADOTROPIN AND PLACENTAL ALKALINE PHOSPHATASE PRODUCTION. (Eng.) Kameya, T. (Natl. Cancer Center Res. Inst., Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan); Kuramoto, H.; Suzuki, K.; Kenjo, T.; Oshikiri, T.; Hayashi, H.; Itakura, M. *Cancer Res.* 35(8):2025-2032; 1975.

1171 EFFECTS OF THYMIDINE ANALOGS UPON GROWTH CONTROL IN CULTURED HORMONE-DEPENDENT RAT)VARY CELLS. (Eng.) Mathews, C. K. (Univ. Arizona, Coll. Medicine, Tucson, Ariz. 85724). *Exp. Cell Res.* 92(1):39-46; 1975.

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1175 MICROVILLI ON CANCER CELLS CAUSE INCREASED AGGLUTINABILITY AND ARE MODULATED BY CYCLIC AMP [abstract]. (Eng.) Willingham, M. C. (Natl. Cancer Inst., Bethesda, Md.); Pastan, I. *Clin. Res.* 23(3):426A; 1975.

1176 THE EFFECT OF CA^{++} METABOLISM REGULATING HORMONES ON GROWTH AND METASTASIZATION OF EXPERIMENTAL TUMORS. (Rus.) Kashulina, A. P. (P. A. Herzen Res. Inst. Oncology, Moscow, U.S.S.R.); Saraeva, Z. M.; Tereschenko, I. P.; Golovanov, M. V.; Briskin, A. I. *Vopr. Onkol.* 21(5):87-91; 1975.

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1183 CELL-SURFACE-ASSOCIATED NUCLEIC ACID IN TUMORIGENIC CELLS MADE VISIBLE WITH PLATINUM-PYRIMIDINE COMPLEXES BY ELECTRON MICROSCOPY. (Eng.) Aggarwal, S. K. (Dept. of Zoology, Michigan State Univ., East Lansing, Mich. 48824); Wagner, R. W.; McAllister, P. K.; Rosenberg, B. *Proc. Natl. Acad. Sci. USA* 72(3):928-932; 1975.

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1187 CORRELATION OF GLUCOCORTICOID RECEPTORS TO THE *IN VITRO* EFFECTS OF CORTISOL ON ^{3}H-URIDINE (^{3}H-U) INCORPORATION INTO RNA IN HUMAN NEOPLASTIC HEMATOPOIETIC CELLS [abstract]. (Eng.) Higby, D. (Roswell Park Mem. Inst., Buffalo, N.Y.); Gailani, S.; Nussbaum, A.; McHugh, M.; Rosen, F. *Clin. Res.* 23(3):339A; 1975.

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1192 ISOENZYMES OF BLOOD SERUM LACTATE DEHYDROGENASE, ESTERASE AND ALKALINE PHOSPHATASE IN SOME BONE TUMORS. (Rus.) Surinov, B. P. (Research Inst. Medical Radiology U.S.S.R. Acad. Medical Sciences, Obninsk, U.S.S.R.); Menzhinskaia, G. V. *Vopr. Onkol.* 21(5):23-26; 1975.

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1199 HEPATOMA, HOST LIVER, AND NORMAL RAT LIVER NEUTRAL LIPIDS AS AFFECTED BY DIET. (Eng.) Wood, R. (Univ. of Missouri Sch. of Medicine, Columbia, Mo. 65201); Falch, J.; Wiegand, R. D. *Lipids* 10(3):202-207; 1975.

1200 HUMAN THYROID CARCINOMA: DIMINISHED TSH RESPONSIVENESS *IN VITRO* COMPARED TO BENIGN "COLD" NODULES AND SURROUNDING NORMAL THYROID TISSUE [abstract]. (Eng.) Field, J. B. (Dep. Med., Univ. Pittsburgh, Pa.); Larsen, P. R.; Kotani, M.; Kariya, T. *Clin. Res.* 23(3):437A; 1975.

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PREFACE

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NOTE

Journal names are abbreviated according to the list of abbreviations used by *Index Medicus*. For rnals not covered by *Index Medicus*, the abbreviations found in *Chemical Abstracts Service Source Index*, 7-1974 Cumulative, are used. New journals are verified in *New Serial Titles* and abbreviated according *International Standard ISO 833*. An asterisk indicates the author to address (other than the primary) in uesting reprints.

LANGUAGE ABBREVIATIONS

Afr.	Afrikaans	Ind.	Indonesian
Ara.	Arabic	Ita.	Italian
Bul.	Bulgarian	Jpn.	Japanese
Chi.	Chinese	Kor.	Korean
Cro.	Croation	Lav.	Latvian
Cze.	Czech	Lit.	Lithuanian
Dan.	Danish	Nor.	Norwegian
Dut.	Dutch	Pol.	Polish
Eng.	English	Por.	Portuguese
Est.	Estonian	Rum.	Rumanian
Fin.	Finnish	Rus.	Russian
Fle.	Flemish	Ser.	Serbo-Croatian
Fre.	French	Slo.	Slovak
Geo.	Georgian	Spa.	Spanish
Ger.	German	Swe.	Swedish
Gre.	Greek	Tha.	Thai
Heb.	Hebrew	Tur.	Turkish
Hun.	Hungarian	Ukr.	Ukrainian
Ice.	Icelandic	Vie.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

A	angstrom(s)	M	molar
ACTH	adrenocorticotropic hormone	mM	millimolar
ADP	adenosine diphosphate	μM	micromolar
AMP	adenosine monophosphate	mOsm	milliosmolar
ATP	adenosine triphosphate	mEq	milliequivalents
BCG	Bacillus Calmette Guerin	min	minute(s)
bid	twice daily	mo	month(s)
C	degrees centigrade	MTD	maximum tolerated dose
cal	calorie(s)	N	normal concentration
kcal	kilocalorie(s)	NAD	nicotinamide adenine dinucleotide
cc	cubic centimeter(s)	NADH	reduced nicotinamide adenine dinucleotide
Ci	curie(s)	NADP	nicotinamide adenine dinucleotidephosphate
mCi	millicurie(s)	NADPH	reduced nicotinamide adenine dinucleotide-phosphate
μCi	microcurie(s)		
cm	centimeter(s)	ng	nanogram(s) (10^{-9})
CNS	central nervous system	od	once daily
cpm	counts per minute	Pa	ambient pressure
dl	deciliter(s)	PAS	periodic acid-Schiff
ml	milliliter(s)	pg	picogram(s) (10^{-12})
μl	microliter(s)	pgEq	picogram equivalent
DNA	deoxyribonucleic acid	po	orally
ED_{50}	median effective dose	ppb	parts per billion
EDTA	ethylenediamine tetraacetic acid	ppm	parts per million
ESR	erythrocyte sedimentation rate	qid	four times daily
g	gram(s)	qod	every other day
kg	kilogram(s)	QO_2	oxygen quotient
mg	milligram(s)	R	roentgen(s)
μg	microgram(s)	RBC	red blood cells (erythrocytes)
Hb	hemoglobin	RNA	ribonucleic acid
hr	hour(s)	sc	subcutaneous
ia	intra-arterial	sec	second(s)
ic	intracerebral	SGOT	serum glutamic-oxalacetic transaminase
icav	intracavitary	SGPT	serum glutamic-pyruvic transaminase
id	intradermal	SRBS	sheep red blood cells
ILS	increased life span	TCD	tissue culture dose
im	intramuscular	TCD_{50}	median tissue culture dose
ip	intraperitoneal	tid	three times daily
ipl	intrapleural	U	unit(s)
it	intratumorous	mU	milliunit(s)
IU	International Unit	UV	ultraviolet
iv	intravenous	vol	volume
K_m	Michaelis constant	WBC	white blood cells (leukocytes)
LD	lethal dose	wk	week(s)
LD_{50}	median lethal dose	wt	weight
m	meter(s)	x	times
mm	millimeter(s)	yr	year(s)

CONTENTS

MECHANISMS BY WHICH CHEMICALS INITIATE CANCER. (Eng.) Farber, E. (Temple Univ. . Med., Philadelphia, Pa.). *J. Clin. Pharmacol.* 1):24-28; 1975.

iscussion concerning the mechanisms by which car-
ogens initiate cancer is presented. Most chem-
l carcinogens are not carcinogenic *per se*, but
: be metabolically converted to chemically reac-
e derivatives. Possible alternate and linear
terns of metabolism are presented. In the alter-
e pattern, as exemplified by 2-acetylaminofluorene,
: of the procarcinogen is converted to ring hydrox-
ted derivatives which are inactive for carcinogen-
ty. A small amount is converted to ultimate car-
ogens by *N*-hydroxylation and esterification. It
ears that the relative levels of activation and
ctivation can be modulated by environmental influ-
es. In the linear pattern, as exemplified by some
ycyclic aromatic hydrocarbons, activation and in-
ivation appear to be different steps in a linear
uence. The activated forms are produced first,
can be further metabolized to inactivated deriv-
ves. The activated derivatives can interact with
y different components in their target cells,
luding DNA, RNA, and protein. It is assumed that
or more of these chemical interactions or physico-
mical effects of the ultimate carcinogen are
ficient for the initiation of carcinogenicity.
is suggested that mechanisms of DNA repair on
ans or cells subject to carcinogenesis be more
ly explored. The nature of the cellular and tissue
nts following initiation are poorly understood
to a lack of means for identifying and isolating
new cell populations and the lack of reproducible
kers. A new marker for preneoplastic and premal-
ant cell populations in liver has been developed.
is suggested that this marker may be of great value
the study of the carcinogenic process. (10
erences)

2 INTEGRATION OF MAMMALIAN, MICROBIAL AND DROSOPHILA PROCEDURES FOR EVALUATING CHEM-
L MUTAGENS. (Eng.) Legator, M. S. (Div. Biol.
. Sci., Brown Univ., Providence, R. I.);
mering, S. *Mutat. Res.* 29(2):181-188; 1975.

development of chemical mutagenesis is discussed,
the use of mammalian, microbial, and *Drosophila*
t systems in testing chemical mutagens is evalu-
d. There is a wide variety of microbial, plant,
ect, and mammalian test systems. In general,
mammalian systems detect intragenic mutations,
le animal systems detect gross chromosomal aber-
ions such as dominant lethals, nondisjunctions,
translocations. *Drosophila* can uniquely demon-
ate all types of genetic alterations. The test
tems are evaluated relative to their ability to
ect mutagenic activity of various compounds;
sphila occupies a unique intermediate position.
is thus recommended that *Drosophila* systems be
d in conjunction with mammalian procedures demon-
ating chemical mutagenesis, while *in vitro* sys-
s are reserved for characterizing the detected
agens. The deficiencies of the *in vitro* activa-
g system (i.e., microsomal preparation) include
lack of the complex dynamic processes occurring in the intact animal, and the lack of alternate routes of potentiation or detoxification. It is suggested that priorities for screening and testing environmental agents for mutagenicity be based on the following criteria: (a) exposure of a large segment of the population, (b) the length of exposure, (c) persistence, and (d) structure-activity relationship. A logical and comprehensive protocol for evaluating chemical mutagens is presented. This protocol describes the selection of chemicals, type of testing, evaluation of results, and decision as to use of the chemical, and strongly recommends the use of *Drosophila*. *Drosophila* is further cited as the only *in vivo* animal test system available capable of cheaply and efficiently detecting the induction of transmitted gene and chromosome alterations in germ cell lines; it can also display delayed effects of chemicals and of indirect acting carcinogens. A discussion of recent progress in mammalian testing methods has noted the development of tester strains of *Salmonella typhimurium*, the modification of the host-mediated assay test, the micronuclei test for cytogenetic analysis, and methods of mutagen detection in blood, urine, and tissue samples.
A novel combined testing program screening approach is described; data thus generated can thus allow a direct correlation of various tests in the same animal and provide detailed analyses of the potential mutagenic activity of the compound investigated. The importance of genetic toxicology and of the use of *Drosophila* procedure is noted. (23 references)

1203 INACTIVATION AND MUTATION INDUCTION IN BACTERIA BY MICROSOMAL METABOLITES. (Eng.) Garner, R. C. (Dept. Experimental Pathology and Cancer Res., Univ. Leeds, 171 Woodhouse Lane, Leeds LS2 3AR, Yorks., U.K.). *Biochem. Soc. Trans.* 3(1):65-69; 1975.

The carcinogenicity of various mycotoxins for rat liver was compared in a microsomal function-bacterial assay. This assay is based on the ability of bacteria to detect electrophilic metabolites of carcinogens and anticancer agents generated during metabolism by microsomal fractions. Flasks containing 250 mg fresh rat liver equivalents of pooled postmitochondrial fractions together with an NADPH-generating system (20 min, 37 C) were incubated with (a) 5 μM aflatoxin B_1, B_2, or sterigmatocystin and *Escherichia coli* AB2480, or (b) 10 μM aflatotoxin G_1 or G_2 and *Salmonella typhimurium* TA 1530. Except for aflatoxin B_2, only those mycotoxins with a 2,3-isolated double bond were converted by liver microsomal fractions into cytotoxic species. None of the compounds at the concentrations used showed any bacterial toxicity in the absence of the liver preparation. Chemical studies have suggested that the active compounds are converted into an epoxide intermediate by mixed-function oxidase attack of the 2,3-isolated double bond and that this is the metabolite responsible for bacterial mutagenicity and probably the animal carcinogenicity. Use of the same microsomal-bacterial assay to study the metabolism of nitrogen-mustard alkylating agents revealed that *p*-hydroxyaniline mustard was more toxic than its parent aniline mustard; the dose that decreased survival to 37% was 1 μg/ml for *p*-hydroxyaniline and 2.7 μg/ml for aniline mustard. Neither the glucuronide of *p*-hydroxyaniline mustard nor *N*-methyl-

N-chloroethylaniline mustard showed any toxicity at these concentrations. This method should enable rapid screening of chemicals for carcinogenicty. Characterization of the cytotoxic metabolites of anticancer drugs could lead to the synthesis of more effective drugs. (22 references)

1204 MYCOTOXINS. (Eng.) Wogan, G. N. (Dept. Nutrition and Food Science, Massachusetts Inst. Technology, Cambridge, Mass. 02139). *Annu. Rev. Pharmacol.* 15:437-451; 1975.

The current status, public health relevance, and illustrative examples of the mycotoxins problem are reviewed. The aflatoxins, the best characterized of the mycotoxins, chemically are highly substituted coumarins containing a fused dihydrofurofuran configuration; two series, the aflatoxin B_1 and G_1 derivatives, are recognized. The aflatoxins are produced by a few strains of *Aspergillus flavus* or *A. parasiticus;* sterigmatocystins are metabolites of *A. versicolor, A. flavus, A. nidulans, A. rugulosum,* a *Bipolaris* species, and a *penicillium.* Despite wide variations in species responsiveness to acute toxicity and carcinogenicity, the liver is the main target organ for aflatoxin B_1. Biochemical changes follow a consistent pattern of DNA and RNA polymerase inhibition. Feces represent the principal excretory route, and radioactivity is retained mainly in the liver. Metabolic transformations of aflatoxin B_1 and G_1 include ring hydroxylations, O-demethylation, epoxidation, and double bond hydration; all are demonstrated semiquantitatively in crude and purified microsomal preparations. Trichothecene mycotoxins (closely related sesquiterpenes) are produced by many species of *Fusarium, Myrothecium, Trichothecium, Trichoderma,* and *Cephalosporium.* A broad spectrum of biological responses is described, with the potency varying considerably with structural modification. Clinical and pathological responses noted include lesions of the oral cavity, gastrointestinal inflammation and hemorrhage, and suppression of rapidly proliferating tissues. Current trends in mycotoxin research include the development and application of new analytical methods, the isolation of fungi from foods, and the identification of the toxic agents produced. Assessment of the public health significance lacks quantitative cause-effect relationships, but suggests several circumstantial associations of mycotoxins with human disease. (66 references).

1205 CROWN GALL TUMORS: ARE BACTERIAL NUCLEIC ACIDS INVOLVED? (Eng.) Drlica, K. A. (Dept. Biochemical Sciences, Princeton Univ., Princeton, N.J. 18540); Kado, C. I. *Bacteriol. Rev.* 39(3): 186-196; 1975.

Experimental evidence bearing on the hypotheses that *Agrobacterium tumefaciens* nucleic acids, phages, or plasmids are involved in plant tumorigenesis is reviewed. Efforts to induce tumors with extracted *A. tumefaciens* nucleic acids, to inhibit tumor formation with nucleases, and to demonstrate transfer of DNA from *A. tumefaciens* to plant cells have collectively produced data consistent with the concept that bacterial nucleic acids play a role in plant tumorigenesis. Two lines of evidence suggest that bacterial genes influence tumor phenotypes: (a) crown gall tumors vary a great deal in their rate of growth, degree of autonomy, and state of differentiation, depending upon the particular bacterial strains used in transformation; and (b) the tumor tissues frequently contain unusual amino acid derivatives depending upon the bacterial strain used to induce the tumor. Genetic experiments have not indicated whether phenotypic differences arise from the continuous production of bacterial gene products by tumor cells or from a self-perpetuating induction of plant genes by the bacterium at the time of transformation. Nucleic acid hybridization has not demonstrated *A. tumefaciens* DNA or RNA in sterile crown gall tissues. *A. tumefaciens* bacteriophages have also been considered as the carriers of the information responsible for transformation. Although purified phages have failed to induce tumor formation, reports claiming that phage DNA base sequences occur in tumor cells and that coliphages can transfer genes to plant cells lend support to the concept. It has recently been shown that plasmids are present in all tumorigenic bacterial strains and are absent in most non-tumorigenic strains of *Agrobacterium.* It is concluded that the hypotheses that *A. tumefaciens* nucleic acids, phages, or plasmids are directly involved in transformation of plant cells are consistent with experimental results, but that there is no strong, unequivocal evidence favoring one hypothesis over another. (101 references)

1206 PRENATAL EXPOSURE TO STILBESTROL AND ADENOCARCINOMA OF THE FEMALE GENITAL TRACT: THE PEDIATRICIAN'S RESPONSIBILITY. (Eng.) Soyka, L. F. (Coll. Med., Univ. Vermont, Burlington). *Pediatrics* 55(4):456-458; 1975.

Data linking *in utero* diethylstilbestrol (DES) exposure to genital tract adenocarcinoma, the nature and extent of risk factors, and approaches to management of girls exposed to DES are reviewed. A registry for cases of clear-cell adenocarcinoma of the genital tract occurring in women under 30 revealed that whereas no history of maternal hormonal therapy was present in 13% of the vaginal and 32% of the cervical cases, the history was positive in 65% and "probable" in an additional 13%. Exposure was prior to 18 weeks of gestation in all cases, and greater than a month's duration in 95%. No apparent tumor relationship is drawn between tumor incidence and dosage. It was suggested that brief exposure, or exposure during the second half of pregnancy, did not constitute a risk. Risk factors of DES daughters are reported as ranging from 4 in 1000 to 1 in 5000. Another study reports a definite increase in adenocarcinoma of the cervix and vagina in all age groups, which is largely unrelated to maternal synthetic estrogens. It is suggested that girls presenting dysfunctional bleeding, intermenstrual spotting and vaginal discharge be examined regardless of DES exposure history. The evaluation was urged to include complete visualization of the vagina and cervix; careful palpation; Papanicolaou smear; biopsy of cervical erosions, suspicious areas, and those not taking up iodine; and prehaps colposcopy. Support for routine examinations is based

the frequent asymptomatic nature at the time of agnosis, and the frequency of vaginal adenosis and her benign lesions found in DES daughters. Where- the occurrence of adenosis is considered by some be a hallmark of *in utero* DES exposure, other udies find cervical erosion to be more common. It concluded that the incidence of genital tract enocarcinoma in young women is sharply increasing, least in part secondary to *in utero* exposure to S. (14 references)

07 CLINICAL AND EPIDEMIOLOGIC CHARACTERISTICS OF YOUNG FEMALES DEVELOPING CANCER AFTER TRAUTERINE EXPOSURE TO DIETHYLSTILBESTROL AND LATED DRUGS. (Eng.) Herbst, A. L. (Dept. Gyne- ology, Massachusetts General Hosp., Boston, Mass.); bboy, S. J.; Scully, R. E. *Proc. Int. Cancer ngr. 11th.* Vol. 2 *(Chemical and Viral Oncogene- s).* Florence, Italy, October 20-26, 1974. Edited Bucalossi, P.; Veronesi, U.; Cascinelli, N. w York, American Elsevier, 1975, pp. 139-145.

ta obtained on 220 cases of genital clear-cell enocarcinoma (submitted to the Registry of Clear- ll Adenocarcinoma of the Genital Tract in Young males) is summarized. Cases were classified as ginal (132) or cervical (88), and were assigned mor stages according to the criteria of the Inter- tional Federation of Obstetricians and Gynecolo- ists. Analysis of the geographic distribution of e birth place of 161 cases showed occurrences roughout the U. S. and in ten other countries. e greatest number of cases appeared in areas ere diethylstilbestrol (DES) or similar compounds re widely used for the treatment of high risk regnancies. Although data support the contention at the risk of development of clear-cell cancer fter intrauterine DES exposure is small, no pre- ise estimate of the risk can yet be calculated. ver 70% of the cases reported positive maternal ormone histories; in all cases in which medication ould be identified, either stilbestrol, dienestrol, r hexestrol were prescribed. Thus far, no data mplicate steroidal estrogens in carcinogenesis. wide variation is found in the dosage of DES ngested by the mothers whose daughters subsequently eveloped cancer; total dosages ranged from less han 500 mg to greater than 15,000 mg. There was lso a great variation in the time that DES therapy as begun. However, in all 102 cases identified, herapy was initiated prior to the 18th wk of ges- ation. The youngest patient with a positive mater- al history of DES therapy was seven years of age t the time of carcinoma diagnosis; the oldest was 7 yr. Approximately 90% of the cases were diagnosed n females 13 yr of age or older; the current average ge of the patients is 17.8 yr. Several forms of reatment are employed. Many stage I and occasional tage II vaginal or cervical carcinomas are treated y radical hysterectomy and/or vaginectomy, with reservation of the ovaries, and replacement of he vagina with a split-thickness skin graft. adiation treatment is also employed. It is con- luded that intrauterine exposure to DES and similar ompounds is associated with the rare development f clear-cell adenocarcinoma of the vagina and ervix in young females. (35 references)

1208 CYCLAMATES: A REVIEW OF THE CURRENT POSITION. (Eng.) Cook, C. E. A. (Abbott Australasia Pty. Ltd., Glenorie, N. S. W., Australia). *Curr. Med. Res. Opin.* 3(4):218-224; 1975.

Evidence leading to the ban on cyclamates as food additives in U.S. and other countries is reviewed. The appeal made against the decision that led to the lifting of the cyclamate restrictions in Australia is also discussed. Two studies led to the prohibition of cyclamates as food additives in U.S. In one experiment, groups of 80 rats were fed a cyclamate/saccharin mixture for two years at levels of 0 mg/kg, 500 mg/kg, 1,120 mg/kg, and 2,500 mg/kg. From the 79th wk to the end of the experiment, half of each treated group was also fed cyclohexylamine hydrochloride at various levels. Eight out of 240 animals developed papillary tumors of the urinary bladder. The author suggests that these results cannot positively identify cyclamates as a carcinogenic agent; the tumors could also have been caused by the saccharin, possible saccharin impurity, cyclohexylamine, bladder calculus, or helminth parasitism. Surviving rats left over after a lengthy interval from an experiment originally designed to study the conversion of cyclamate to cyclohexylamine were used in the second experiment leading to cyclamate restriction. In the original experiment, groups of 20 weanling rats were fed synthetic diets either low (10%) or high (20%) in protein and containing 0%, 1.0%, or 2.0% calcium cyclamate for 15 mo. At autopsy, none of the surviving rats showed evidence of bladder cancer or other malignancy. Additional groups of 20 weanling rats were fed over a 2-yr period on Purina rat chow diets containing 0%, 0.4%, 2.0%, or 10% calcium or sodium cyclamate. Intermittently, ^{14}C-cyclamate was used. At autopsy, carcinomas were found in the bladders of three rats fed the ^{14}C-calcium cyclamate diet. Since the cyclamate ban was issued, 20 long term carefully planned and adequately controlled experiments testing the carcinogenic potential of cyclamates and cyclohexylamine in rats, mice and hamsters have proved negative. These results have led to a rescinding of precautionary restraints on the use of cyclamates in Australia. (16 references)

1209 COMPOSITION OF MEMBRANES OF CELLS TRANSFORMED BY TUMORIGENIC DNA AND RNA VIRUSES. (Eng.) Brady, R. O. (Natl. Inst. Neurol. Dis. Stroke, Bethesda, Md.). *Am. J. Clin. Pathol.* 63(5): 685-694; 1975.

A review of information is presented on the composition of cell membranes transformed by tumorigenic DNA and RNA viruses. The ganglioside content of membranes have been altered, independent of cell type, by simian virus 40 (SV40) or polyoma virus; only the smallest ganglioside, monosialosyllactosylceramide (G_{m3}) is present in transformed cells. Cells transformed by Kirsten strain of murine sarcoma virus have both G_{m3} and G_{m2} ganglioside. Two possible biochemical mechanisms are: excessive rapid catabolism of higher gangliosides, and a block in the enzymatic synthesis of gangliosides. The two anabolic enzymes most affected by the virus are N-acetylgalactosaminyltransferase and galactosyltransferase. The genome of the tumorigenic virus

must be stably inserted into the DNA of the host cell to cause the enzyme and structural modification. The precise role of gangliosides in abnormal social behavior of transformed cells is not clear. Gangliosides may play a direct or indirect role by masking or unmasking other groups or may play a critical role in altered growth properties of the transformed cells. (20 references)

1210 VIRUSES IN THE TRANSMISSION OF CANCER. (Eng.) Kalter, S. S. (Microbiol. Infect. Dis., Southwest Found. Res. Educ., San Antonio, Tex.); Heberling, R. L.; Hellman, A.; Todaro, G. J.; Panigel, M. *Proc. R. Soc. Med.* 68(3):135-140; 1975.

Recent findings on the presence of C-type viruses in normal primate placentas is reviewed, and evidence suggesting vertical transmission of the virus presented. Oncogenicity is demonstrated for the DNA viruses, including adeno-, herpes-, papova-, and pox-viruses; there is a questionable association of RNA tumor viruses with primate tumors. The presence of endogenous type-C particles in simian and human placentas derived from apparently normal sources is reported. The number of particles varied according to species; the baboon and rhesus monkey placentas consistently contain the most, and human placenta contains the least. No predilection for racial groups or number of pregnancies is apparent. Examination of preimplantation stages of embryonic development reveals immature C-type particles, but no budding forms. While not all species are found to contain C-type particles, their presence is also demonstrated in nonprimate placentas. The placental C-type agents have been successfully isolated and cultured; however, placental cultures from several simian species demonstrate viral cytopathology due to foamy-virus with somewhat similar morphology to C-type virus particles. The data provide evidence for the presence of a primate C-type virus in normal primate tissue. Assays suggest that the baboon isolate is distinctly different from previously described mammalian type-C viruses; the baboon agent is also endogenous and "switched on" only in the baboon placenta. Although the failure to detect C-type viral particles in fetal tissue is in contrast to other reports, this may be a function of the number of specimens reviewed. (31 references)

1211 PROPERTIES OF ONCORNAVIRUS STRUCTURAL COMPONENTS AND THEIR EXPRESSION IN THE HOST CELL. (Eng.) Bolognesi, D. P. (Duke Univ. Medical Center, Dept. Surgery, Durham, N. C.); Leis, J. P.; Grant, J. P.; Schäfer, W. *Proc. Int. Cancer Congr. 11th.* Vol. 2 *(Chemical and Viral Oncogenesis).* Florence, Italy, October 20-26, 1974. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 176-181.

Virion constitutents (apparently) involved in the development and maintenance of certain forms of virus-induced neoplasia *in vivo* are discussed. The oncornavirus genetic information is contained in RNA that sediments with a coefficient of 60-70S in neutral sucrose gradients. Results of denaturation studies suggest that the virus RNA possesses a great deal of secondary structure, and electron microscopic examination reveals a network structure of several strands held together at many points. Several lines of investigation suggest that the subunits of the RNA may be identical (polyploid model). However, a haploid model, suggesting that the RNA pieces are unique or largely so, is also proposed. An alternative model for the complexity of 34S RNA suggests that avian tumor virus 34S RNAs contain redundant sequences and unique sequences, resulting in a complexity of greater than 3×10^6. A summary of the general properties of the structural components of murine C-type viruses notes that three components possess interspecies reactivity, that polypeptide p12 is highly type-specific, and that p10 is primarily group-specific. Further evidence has indicated that glycoproteins (gp)71, gp45 and protein (p)15 are the primary species to which free natural antibody in the mouse can be measured. The expression of endogenous virus information is studied in both avian and mammalian systems. Three types of normal avian cells, differing in the expression of the major external virus glycoprotein (gp85), have been found; results suggest that the expression of gp85 and p27 is noncoordinated. The analogous molecules of mammalian viruses (gp71 and p30) are found expressed both coordinately and noncoordinately. Analyses of the viral structural antigen by immunofluorescence and serum cytotoxicity indicate that p30 may arrive at the cell surface from without and suggest that the primary antigenic determinant in the cytotoxic reaction is a virion surface component. The studies reviewed thus indicate that RNA tumor virus structural and nonstructural components can appear on the surface of murine cells harboring the virus genome, and that gp71 and p70 possess both species and interspecies determinants. (32 references)

1212 A REVIEW OF PRIMATE HERPES VIRUSES. (Eng.) McCarthy, K. (Dep. Med. Microbiol., Univ. Liverpool, England); Tosolini, F. A. *Proc. R. Soc. Med.* 68(3):145-150; 1975.

The 37 primate herpes virus strains known to exist are reviewed, and an interim classification system is presented. Although there are no generally accepted rules for naming new virus isolates, use is made for purposes of classification of the nature of the disease or subclinical infection in the primary host; or more often in the secondary host in which the disease first became manifest. Four groups, based primarily on disease patterns in primates, are discussed. Viruses causing neurological or generalized disease, typified by *Herpesvirus hominis* I and II, include a wide variety of virus strains; no antigenic similarity appears to exist between the Old World and New World strains. Viruses causing exanthematous diseases constitute the second group. All of these viruses cause diseases resembling human chicken pox, with a wide variation in virulence. Two subgroups are recognized: host range restrictive viruses causing nonlethal infection, and nonspecies specific

ruses responsible for severe illness and high mor-
lity. Infections caused by cytomegalovirus (CMV)
e commonly silent postnatally. Although CMV are
nerally species specific *in vitro*, vervet CMV grows
adily in human embryonic fibroblasts. The proto-
pe of the fourth group, viruses causing benign
malignant lymphoproliferative disease, is the
stein-Barr virus. A fifth, little studied group
cludes viruses not known to cause disease. The
ofusion of recent virus isolations is attributed
two factors: use of primate tissues in cell cul-
res revealing latent agents, and accidental cross-
fection of primates producing clinical illness.
l herpes virus infections include the possibility
virus latency. While only few have been shown
cogenic, further investigations may reveal a se-
ctive capacity for inducing malignancy in appro-
iate host species. (68 references)

213 CAT LEUKEMIA VIRUS AND IMMUNOLOGY. (Eng.) Deinhardt, F. (Rush-Presbyterian St. Lukes edical Center, Chicago, Ill. 60612). *Blood* 46(1): 43-146; 1975.

n international symposium was held to assess cur-
ent knowledge of feline leukemia virus (FeLV) and
eline sarcoma virus (FeSV) and the relevance of
tudies of these viruses to leukemia in other spe-
ies, including man. The structure of FeLV and Fe-
V is similar to other RNA C-type viruses; the nu-
leic acid of the feline viruses is a 60-70S RNA,
nd the subunits have a molecular wt between 2
nd 2 x 10^6 daltons. Some endogenous RD-114-like
eline viruses have RNA of only 56S and appear to
e smaller than conventional FeLV viruses by elec-
ron microscopy. FeLV and FeSV differ from other
ype-C viruses in that they frequently occur as an-
igenic mixtures of viral subgroups A, B, or C. Vi-
uses of subgroup B have the widest host range, cau-
ing productive infection of cells in a variety of
ammalian species. Under natural conditions, in-
ection with FeLV and FeSV occurs horizontally, and
here is no proof for true vertical transmission of
he viruses. Infection is often silent, and a per-
istent carrier state and disease develop in less
han 0.1% of infected animals. Animals with feline-
ncornavirus-associated cell-membrane antigen (FOCMA)
ntibody titers of 32 or above are relatively resis-
ant to tumor induction. Immunization with FeLV-
nfected cells (and, to a lesser extent, with virus)
ill induce FOCMA antibodies, and animals thus im-
unized become resistant to superinfection and tu-
or induction by FeLV/FeSV. It is emphasized that
he vertical transmission reported for other animal
ncornaviruses has occurred mainly in studies using
ighly inbred animals. In contrast, the feline dis-
ase occurs in outbred populations, a situation more
omparable to natural conditions in animals a.. man.
he demonstration that the feline leukemia sarcoma
yndrome is widespread, but that less than 0.1% of
nfected animals develop a persistent carrier state
nd disease, has implications for the epidemiology
f the human disease. The distribution of antibodies
n cat populations must also be considered when in-
erpreting data on the distribution of antibodies to
ntigens found in human tumors. (No references)

1214 EXCITEMENT OVER β_2-MICROGLOBULIN. (Eng.) Raff, M. C. (No affiliation given). *Nature* 254(5498):287-288; 1957.

Observations suggesting an evolutionary link between β_2-microglobulin, immunoglobulin (Ig), and the major histocompatibility (H) antigens are presented. The complete amino acid sequence of human β_2-microglobulin shows a striking degree of homology with the constant domains of human Ig. The findings suggest that β_2-microglobulin represents a free Ig domain with effector function similar to the terminal domain of IgG. Two independent lines of evidence indicate that β_2-microglobulin is an integral part of the subunit structure of all the membrane proteins which carry the serologically defined major histocompatibility antigens on the surface of all normal nucleated cells. The light chain of HL-A appears identical with β_2-microglobulin, which noncovalently binds to the heavy chains in a structure similar to Ig. Studies employing the binding of multivalent ligands to specific determinants on cell surfaces reveal that the association of solubilised HL-A with β_2-microglobulin is not an artifact of the solubilization or separation procedures. The structure of β_2-microglobulin suggests it is found in the membrane only through interacting with other membrane proteins; it is found associated with thymus leukemia antigens and teratocarcinoma alloantigens, both of which are determined by genes closely linked to the H-2 complex. While not true for all alloantigens coded for by genes in the H-2 complex, it is possible that the β_2-microglobulin serves to stabilize the functional conformation of the membrane polypeptides. Despite the apparent lack of genetic linkage, homology in structure between β_2-microglobulin and some Ig domain suggests the possibility of evolution from a common ancestral gene. The physical association of β_2-microglobulin with some of the T cell receptor proteins suggests an evolutionary link between B-cell-mediated humoral immunity and T-cell-determined cell-mediated immunity. The possible immunological importance is extended by recent findings that anti-β_2-microglobulin antibodies inhibit various T cell responses, although the true function of β_2-microglobulin remains unknown. (19 references)

1215 CROHN'S DISEASE: PRECANCEROUS CHANGES IN COLITIS. (Eng.) Morson, B. (St. Mark's Hosp., London, England). *Dis. Colon Rectum* 18(3): ?.7-208; 1975.

The interpretation of precancer in ulcerative colitis is reviewed. A study on colectomy specimens shows the value of rectal biopsy in revealing epithelial changes that antedate the development of cancer in chronic ulcerative colitis. Use of the colonoscope is recommended in taking multiple biopsies from the entire large bowel for the purpose of assessing the extent of any precancerous epithelium. Signs of severe epithelial atypia or dysplasia are regarded as predisposing to cancer, although carcinoma *in situ* does not necessarily progress to cancer. The precancerous state in ulcerative colitis involves the distal large bowel most frequently. Although it occasionally involves limited areas of

the colon, it is mostly diffuse. The concept of a phase of epithelial atypia, usually in a flat mucous membrane, is generally accepted. The complication of cancer in ulcerative colitis is uncommon. However, more in depth study of the different grades of epithelial change, and the determination of clinical significance is recommended.
(No references)

1216 THYROID CARCINOMA (Eng.) DeGroot, L. J. (Dept. Medicine, Univ. Chicago, Chicago, Ill. 60637). *Med. Clin. North. Am.* 59(5):1233-1246; 1975.

The etiology, pathology, presentation, diagnosis, and management of thyroid carcinoma are reviewed. Strong data link the occurrence of thyroid cancer to previous exposure to external radiation, radioactive iodide, and radiation fall out. The two-fold radiation effects observed are nuclear damage and decreased cell metabolic function; a latent period of 10-20 yr is noted. Malignancies are associated with prolonged thyroid stimulating hormone (TSH) exposure, parathyroid adenomas, and multinodular goiter. The following pathological classification of the thyroid tumors is acknowledged: differentiated tumors (papillary adenocarcinoma, mixed papillary and follicular carcinoma, follicular carcinoma, follicular adenocarcinoma, medullary carcinoma); undifferentiated tumors (small-cell, giant-cell, carcinosarcoma); and miscellaneous tumors (lymphoma, squamous cell epidermoid carcinoma, fibrosarcoma, metastatic tumor). The usual clinical presentation of the thyroid tumor is as an incidentally discovered neck lump; an enlarging painful mass, dysphagia, dysphonia, dyspnea, or metastatic nodules are also occasionally presented. The course of papillary, follicular, Hurthle cell, medullary, anaplastic, and undifferentiated tumors is discussed in depth. Diagnostic techniques employed include: prior histories of X-ray exposure, recent growth, or familial thyroid disease; X-ray examination; (elevated) serum thyroglobulin levels; diminished radioactive iodide accumulation; fluorescent thyroid scanning; fine needle aspiration cytology; aspiration of cysts; and needle biopsy. Clinical management and possible therapeutic approaches are highly dependent on the patient's age and the stage of the disease. In Stage I, intrathyroidal, differentiated papillary and follicular cancers, a near-total thyroidectomy is favored. Stage II thyroid cancers, with movable cervical metastases, are managed by thyroidectomy and modified neck dissection. Stage III tumors with direct local invasion or fixed cervical nodes are treated with total thyroidectomy and near dissection, while Stage IV patients require individual management. (30 references)

1217 LEUKEMIA IN SURVIVORS OF WILMS TUMOR. (Eng.) Miller, R. W. (Natl. Cancer Inst. Bethesda, Md. 20014). *J. Pediatr.* 87(3):505-506; 1975.

Leukemia in five survivors of Wilms tumor who had been treated with chemotherapy and radiation may be attributed to the radiation therapy. This brief review cites evidence that in survivors of the atomic bomb and other persons exposed to ionizing radiation, this agent induces leukemia in the same time interval (4-13 yr after therapy for Wilms tumor) and with the same histologic array as was observed in these five children. The leukemogenic role of actinomycin D, given to 3 of 5 children, is not known at present. The possibility also exists that some children with Wilms tumor are naturally predisposed to leukemia and to other second primary cancers. Among children with Wilms tumor, high risk may be signified by bilateral neoplasms, familial occurence, hemihypertrophy, hamartomas, genitourinary abnormalities, or aniridia. Among the five patients, one had hemihypertrophy and another had multiple large pigmented nevi (a hamartomatous disorder). It is suggested that the risk of leukemia among survivors of Wilms tumor may be diminished as it is learned how little radiotherapy is required to achieve a cure. Future studies may also reveal whether or not survivors who develop leukemia differ from normal individuals in their personal or family histories; if so, therapy and prognosis must be tailored accordingly. (13 references).

1218 SYMPOSIUM NO. 18: ONCOGENETICS--GENETICS OF HUMAN CANCER. (Eng.) Knudson, A. G., Jr. (Univ. Texas Health Science Center at Houston, Houston, Tex. 77025). *Genetics* 79(Suppl.):305-316; 1975.

Heritable human cancers are related to general human cancer and current notions of environmental carcinogenesis. The clearest cases of heritable cancer, in which Mendelian dominant inheritance with high penetrance is operative, are: neurofibromatosis of von Recklinghausen, polyposis of the colon, retinoblastoma of childhood, and hereditary adenocarcinomatosis. The dominantly inherited gene of polyposis invariably produces polyposis and adenocarcinoma of the colon; the carcinoma develops much earlier than in the general population, but is histologically the same. Hereditary adenocarcinomatosis also exhibits an earlier-than-usual onset and multiple primary tumors histologically indistinguishable from those of the nonhereditary form, as does retinoblastoma. A two-step model is presented for the development of both hereditary and nonhereditary retinoblastoma: (1) a germinal mutation, followed by (2) a somatic mutation. Such a two-step model is also suggested for Wilm's tumor of the kidney, neuroblastoma, other childhood tumors, adult pheochromocytoma, and gastric carcinoma. Genetic states found to impose susceptibility to cancer include aneuploidy (trisomy for chromosome 21), D-deletion, and the Philadelphia chromosome. Structural chromosomal abnormalities associated with leukemia and cancer in the recessively inherited chromosome breakage syndromes of Fanconi and Bloom suggest they may be a fundamentally different process from other cancers. Three possible relations of dominant mutations with chromosome breakage are suggested. It is also suggested that such genetic disorders predispose susceptibility to the environmental agents of viruses, radiation, and chemical carcinogens. Cancer development is suggested to be due to the possible interactions of genetic predisposition, metabolic pattern, tumor virus, radiation, and other intrinsic and extrinsic factors. (46 references).

19 THE PROXIMATE CONTROL OF TRANSCRIPTION IN NORMAL AND NEOPLASTIC CELLS. (Eng.) Anerson, K. M. (Faculty of Medicine, Banting Inst., 0 College St., Toronto, Canada); Guzik, G. *Int. Biochem.* 6(4):231-262; 1975.

e literature on the control of transcription in rmal and neoplastic cells is reviewed. The fortion of tumors is discussed in terms of impaired d inappropriate differentiation (or 'disdifferentiation', e.g., ectopic formation of hormones by variety of tumors). Two mechanisms are outlined r the ectopic synthesis of an ACTH-like protein bronchogenic and oat cell carcinomas of the lung. cancer, disordered cellular differentiation is ccompanied by a qualitatively and quantitatively tered distribution of RNA molecules. There is rowing evidence for antagonistic effects of cyclic cleotides on replicating and nonreplicating cells, d on their role in the pleiotypic control of cells culture. Reciprocal effects of cyclic nucleotides specific RNA polymerases located at different inranuclear sites, possibly mediated by proteins that ind cyclic nucleotides, could provide a physical asis for the proximate control of transcription asciated with certain aspects of normal and abnormal ell growth and differentiation. However, such factrs may represent only one example of a more general nctional category of proteins regulating transcripion in eukaryotes. Not all of these factors need teract with low-molecular-wt compounds. In tumors, e existence of qualitatively inappropriate, quanitatively insufficient, or functionally abnormal reulatory proteins may provide part of the explanation r their differing spectrum of RNA molecules. Quaitatively- or quantitatively-impaired regulatory roteins might include protein kinase subunits that rmally bind cyclic nucleotides, mammalian sigma-like roteins with functions resembling bacterial sigma intiation proteins, or proteins that alter the conforation of a particular region(s) of chromatin. Whether factors affecting cell replication might alter the vailability of template, increase the affinity of a olymerase for specific sites, alter subsequent RNA hain elongation, or modify RNA synthesis by some ther mechanism remains to be determined. (197 reerences)

220 MUTATIONS AND DNA REPLICATION. (Eng.) Bautz, E. K. F. (Institut für Molekulare enetik der Universität, Berliner Strasse 15, 69 eidelberg (W. Germany)). *Mutat. Res.* 29(2):189-93; 1975.

he effects of point mutations on DNA replication re discussed, and a biochemical explanation is ffered for the unusually high precision with which NA is replicated. It is assumed that some genes re more mutable than others; the rIIA gene and IIB gene mutants are cited as well-documented examles of this. Comparative forward mutation rates re tabulated and a comparison of *Neurospora crassa* nd *Drosophila* mutation rates suggests that most stimates of spontaneous mutation rates are high. n addition, a fair proportion of morphological utant characters are produced by more than one ene, so that division of the mutant frequency by the number of base pairs coding an average polypeptide does not give an accurate estimate of mutation rate per base-pair replication. In determining the specificity of DNA replication, a minimum error frequency of 10^{-6} is suggested on thermodynamic grounds. In a discussion of the synthetase and exonuclease activities of DNA polymerase I, an error frequency of 10^{-12} is suggested. The use of RNA primers for the DNA replication is also discussed. Although it is supposed that the only mutations that count are those occurring in the germ line between one generation and the next, accumulated somatic mutations may interfere with longevity. Assuming that there are 10^{14} cells in the adult human, and that there is a mutation rate of 10^{-11} per base pair replication, at least 10^{12} mutations are carried for any given gene. A recessive "oncogene" is suggested. The incidence of cancer for each cell could be as high as 10^{-16}/cell replication, and the possibility of spontaneously acquiring cancer during a man's lifetime would then be higher than 0.1. It is concluded that somatic mutations accumulate in large numbers during the lifetime of an organism, usually without harm, unless the mutation leads to a phenotype, as in cancer, where the mutant cells outgrow the wildtype cells. (7 references)

1221 CHARACTERISTICS OF HETEROGENEOUS NUCLEAR RNA IN NORMAL SMALL LYMPHOCYTES AND IN ACUTE LEUKEMIA BLAST CELLS: AN OUTLINE. (Eng.) Torelli, U. (Inst. Medical Pathology, Univ. Modena, I-41100 Modena, Italy). *Acta Haematol. (Basel)* 54(4):234-241; 1975.

The characteristics of heterogeneous nuclear RNA (hnRNA) of normal small lymphocytes and of leukemic blast cells are reviewed. The average lifetime of the molecules of this RNA class in both types of cell is much longer than that so far reported for rapidly proliferating cells. In both types of cell the newly synthesized RNA includes rapidly hybridizing sequences. There is evidence, although circumstantial, that in leukemic cells these sequences are markedly different from that of normal lymphocytes. In both normal lymphocytes and leukemic blast cells the rapidly hybridizing RNA sequences are in part involved in secondary structure. A significant fraction of the hnRNA appears in RNase-resistant form with double-stranded properties. About one fifth of the hnRNA molecules of normal lymphocytes carries poly(A) segments, whereas this proportion is markedly higher in leukemic cells. It is postulated that accumulation of double stranded, long-living RNA molecules is a critical process in limitation of growth of both normal lymphocytes and leukemic cells. (31 references)

1222 PLACENTAL PROTEINS AND THEIR SUBUNITS AS TUMOR MARKERS. (Eng.) Rosen, S. W. (Natl. Inst. Arthritis Metab. Dig. Dis., Bethesda, Md.); Weintraub, B. D.; Vaitukaitis, J. L.; Sussman, H. H.; Hershman, J. M.; Muggia, F. M. *Ann. Intern. Med.* 82(1):71-83; 1975.

The presence of human placental lactogen (HPL) and human chorionic gonadotrophin (HCG) in males and

nonpregnant females was studied retrospectively and related to the presence of neoplasm. By using Sepharose columns with antibody to HPL, detection by immunoassay was increased to 2 pg HPL/ml plasma. No HPL was detected in any of 78 normal volunteers, nine patients with benign tumors, or 194 patients with non-neoplastic disease. Detectable levels of 1.0 μg HPL/ml or greater was found in 16 of 295 patients with nontrophoblastic cancers (5/187 with lung, 2/15 with liver, 1/8 lymphoma and leukemia, and 7/85 with other cancer). Immunoreactive HCG (including HCG and its subunits) was detectable in patients with cancer of the lung (13/147), ovary (5/12), gastrointestinal tract (62/135), and in lymphomas (5/251) and melanoma (9/98). Ectopic secretion of HCG associated with gastrointestinal cancer was most significant in pancreatic tumors (14/42), adenocarcinoma of the stomach (16/73) and hepatomas (14/82). A 46-yr-old man with bronchogenic cancer had levels of 1800-2400 μg/ml HCG prior to therapy. After radiotherapy (3,600 rads) and chemotherapy (cyclophosphamide, dactinomycin and vincristine), the tumor shrunk radiographically and symptoms of vena caval obstruction improved. HCG levels had declined to 200 μg/ml. The nodule again grew and HCG levels rose to 53,000 μg/ml just prior to death. HCG and HPL are valuable tumor markers; when they are present, changes in serum levels appear to correlate with tumor activity changes. (78 references)

1223 SMOKING AND DISEASES OF THE UPPER RESPIRATORY TRACT. (Ger.) Matzker, J. (HNO-Klinik der Stadt Koln, 5 Koln 80, Neufelder Strasse 32, West Germany). *Fortschr. Med.* 93(9):449-452; 1975. (No references)

1224 TOBACCO SMOKING AS PRINCIPAL FACTOR FOR BRONCHITIS, EMPHYSEMA AND LUNG CANCER. (Ger.) Gsell, O. (CH-9000 St. Gallen, Zwinglistrasse 21, Switzerland). *Fortschr. Med.* 93(9):445-449; 1975. (9 references)

1225 ORAL CONTRACEPTIVES. (Ger.) Beck, A. (II. Universitats-Frauenklinik, Spitalgasse 23, A-1090, Vienna, Austria). *Wien. Med. Wochenschr.* 125(32-35/Suppl. 3):29-37; 1975. (48 references)

1226 MUTAGENICITY TESTS OF FOREIGN SUBSTANCES (MUTAGENITAETSPRUEFUNG VON FREMDSTOFFEN). (Eng.) Ehling, U. H. (No affiliation given). 27 pp., 1974. [available through National Technical Information Services, Washington, D.C. Document No. PB-237 803-T/GA]

1227 VIRUSES, EVOLUTION AND CANCER. BASIC CONSIDERATIONS. PROCEEDINGS OF A CONFERENCE, MONTREAL, AUG. 1973. (Eng.) Edited by Kurstak, E. (No affiliation given); Maramorosch, K. New York, Academic Press, 1974, 814 pp.

1228 THE INS AND OUTS OF RNA TUMOUR VIRUSES. (Eng.) Lewin, R. (No affiliation given). *New Sci.* 68(969):22-23; 1975. (No references)

1229 DOES THE CLASSIC TUMOUR VIRUS LIVE IN HUMAN TOO? (Eng.) Anonymous. *New Sci.* 68(969):6; 1975. (No references)

1230 FELINE LEUKAEMIA VIRUS AND ITS CLINICAL EFFECTS IN CATS. (Eng.) Mackey, L. (Dep. Vet. Pathol., Univ. Glasgow, Scotland). *Vet. Rec.* 96(1):5-11; 1975.

1231 CHEMICAL INDUCTION OF CANCER. VOLUMES IIA AND IIB. (Eng.) Arcos, J. C. (No affiliation given); Argus, M. F. New York, Academic Press, 1974, 385 pp. and 379 pp.

1232 POOL OWNERS IGNORE CANCER WARNING. (Eng.) Anonymous. *New Sci.* 67(964):490; 1975. (No references)

1233 RENEWED CONCERN ABOUT RUBBER ANTI-OXIDANTS. (Eng.) Anonymous. *New Sci.* 67(968):694; 1975. (No references)

1234 STUDY OF THE METABOLISM OF CROWN-GALL TISSUE GUANIDINES BY *AGROBACTERIUM TUMEFACIENS* T_{37} STRAIN. (Fre.) Petit, A. (Physiologie Vegetale, INRA, 78000 Versailles, France); Tempe, J. *C. R. Acad. Sci. (D) (Paris)* 281(5,6,7,8):467-469; 1975. (15 references)

1235 THE GENUS *AGROBACTERIUM* AND PLANT TUMORIGENESIS. (Eng.) Lippincott, J. A. (Dept. Biological Sciences, Northwestern Univ., Evanston, Ill. 60201); Lippincott, B. B. *Annu. Rev. Microbiol.* 29:377-405; 1975. (246 references)

1236 MICROSOMAL METABOLISM AS A DETERMINANT OF AFLATOXIN TOXICITY. (Eng.) Tilak, T. B. G. (Natl. Inst. of Nutrition, Indian Council of Medical Res., Hyderabad 500007, India); Nagarajan, V.; Tulpule, P. G. *Experientia* 31(8):953-954; 1975. (32 references)

1237 HAIR DYES AND CANCER. (Eng.) Anonymous. *Lancet* 2(7927):218; 1975. (No references)

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1239 THE ETIOLOGY OF HUMAN BREAST CANCER. ENDOCRINE, GENETIC, VIRAL, IMMUNOLOGIC AND OTHER CONSIDERATIONS. (Eng.) Papaioannou, A. N. (No affiliation given). New York, Springer-Verlag, 1974, 216 pp.

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1244 LYMPHORETICULAR PROLIFERATIVE DISORDERS OF THE CNS AND OTHER ORGANS: ANALOGIES AND DIFFERENCES. (Eng.) Kepes, J. J. (Univ. of Kansas Medical Center, Kansas City, Kans. 66103); Kepes, M. *Acta Neuropathol.* [*Suppl.*] *(Berl.)* 6: 75-79; 1975. (12 references)

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1246 THE SIGNIFICANCES OF EXPERIMENTAL GLIOMAS FOR HUMAN DISEASE. (Eng.) Zimmerman, H. M. (No affiliation given). *Recent Results Cancer Res.* 51:6-19; 1975. (27 references)

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1248 MALIGNANT LYMPHOMAS OF THE DIGESTIVE TRACT. (Fre.) Tabbara, W. S. (Service d'Anatomie Pathologique, Hopital Hotel-Dieu de France, Beirut, Lebanon). *J. Med. Liban.* 28(2): 187-192; 1975. (20 references)

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1253 11TH INTERNATIONAL CANCER CONGRESS, FLOR-ENCE, OCTOBER 20-26, 1974. REPORT ON EPIDEMIOLOGICAL AND PROPHYLACTIC ASPECTS. (Ger.) Abelin, T. (Institut fur Social- und Praventiv-medizin der Universitat Bern, Switzerland). *Cancer Inf.* 10(2):39-46; 1975. (No references)

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1255 THE CELL CYCLE IN MALIGNANT AND NORMAL TISSUES. (Eng.) Mendelsohn, M. L. (Bio-medical Div., Lawrence Livermore Lab., Univ. Cali-fornia, Livermore, California). *Cell Cycle in Malig. Immun., Proc. Annu. Hanford Biol. Symp., 13th.* Rich-land, Washington, D. C., U.S. Energy Research and Development Administration, 1975, pp. 293-314. (40 references)

1256 MITOTIC CONTROL IN THE BODY. (Eng.) Cam-eron, I. L. (Univ. Texas Health Science Cent. at San Antonio, San Antonio, Tex.). *Cell Cycle in Malig. Immun., Proc. Annu. Hanford Biol. Symp., 13th.* Richland, Washington, D. C., U.S. Energy Re-search and Development Administration, 1975, pp. 76-103. (61 references)

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1258 WHAT TURNS CELLS ON? (Eng.) Lewin, R. (No affiliation given). *New Sci.* 67(968): 712-714; 1975. (No references)

1259 STEROID ACTIVITY OF NORMAL AND NEOPLASTIC BREAST TISSUE. (Eng.) Skelley, D. S. (Baylor Coll. of Medicine, Houston, Tex.); Besch, P. K. *Clin. Obstet. Gynecol.* 18(2):239-252; 1975. (43 references)

260 CHARACTERIZATION OF MONOSOMES PRODUCED BY AFLATOXIN B_1. (Eng.) Hayes, L. C. (Univ. nsas Med. Cent., Kansas City, Kans. 66103); Plapp, V.; Tilzer, L. L.; Chiga, M. *Chem. Biol. Interact.*)(5):343-347; 1975.

elucidate the mechanism by which aflatoxin B_1 disgregates liver polysomes, the monosomes produced by latoxin B_1 were studied. Male F-344 rats were incted i.p. with 1.5 mg/kg aflatoxin B_1 and sacrificed hr later. The livers were homogenized and fraconated to obtain ribosome pellets from the postmitoondrial supernatant. The 80S monosome peak was isoted to determine the transfer RNA (tRNA) content of e aflatoxin B_1-produced monosomes. RNA (350 μg) s layered on polyacrylamide gels, electrophoresed, d the 4S and 5S RNA peaks were traced at 260 nm in Gilford spectrophotometer. A single dose (1.5 mg) aflatoxin B_1 produced 70% disaggregation of rat ver polysomes into monosomes. After centrifugation, e 80S monosomes dissociated into 40S and 60S ribomal subunits containing 0.3 M KCl and therefore cking messenger RNA. The monosomes produced by latoxin B_1 contained 0.6 molecule of tRNA and/or inoacyl tRNA but no peptidyl tRNA, similar to monomes produced by 72-hr starvation. Inhibition of ssenger RNA synthesis could account for decreased mbers of liver polysomes. The observations that e monosomes produced by aflatoxin B_1 are dissocble into subunits and lack peptidyl tRNA indicate at they are runoff ribosomes.

261 CIRCUMSTANCES ASSOCIATED WITH THE CONTAMINATION OF FOOD BY AFLATOXIN IN A HIGH PRIARY LIVER CANCER AREA. (Eng.) Van Rensburg, S. J. Natl. Res. Inst. Nutritional Diseases South African edical Res. Council, Parowvallei, CP, South Africa); irsipuu, A.; Coutinho, L. P.; Van der Watt, J. J. *Afr. Med. J.* 49(22):877-883; 1975.

ifferences in living habits that contribute to the ntake of aflatoxin by a population in Inhambane, ozambique were studied. Sample collection clusters ere chosen at random in six sub-districts, and equal umbers of samples were made over a 3-yr period. he samples consisted of dry food and grain collected efore processing and of "plate" samples collected efore consumption. Defects found in food producion, harvesting, storage and preparation contribted to fungus growth. Analysis of dry stored food vailable to households revealed highest aflatoxin ontamination in 153 samples of groundnuts (1,036.0 g/kg). Contamination can be reduced by cooking and ot eating left-over food, because refrigeration is acking. Groundnuts are not usually roasted (a procss known to reduce aflatoxin content). Aflatoxin s known to be produced at temperatures from 13-40 C nd humidity over 85%. Insect damage to the pods ccurs while they are still in the soil, and is assoiated with aflatoxin contamination. Because droughts ncourage insect damage, which results in inoculaion with aflatoxin-producing *Aspergillus flavus* pores, groundnuts from irrigated land are relatively ree of damage. The best method for drying is to andpick the pods and dry them in a single layer in he sun away from the ground. Moisture below 10% prevents insect attack during storage, and clean jute bags are better than tins. The pods should remain whole during storage, so that fungal spores may not enter. Education is the best way to help reduce the intake of high concentrations of aflatoxin. Diversification of the diet is also recommended.

1262 EXPOSURE TO ASBESTOS AND LARYNGEAL CARCINOMA. (Eng.) Stell, P. M. (E.N.T. Infirm., Liverpool, England); McGill, T. *J. Laryngol. Otol.* 89(5):513-517; 1975.

One hundred and nineteen patients with squamous carcinoma of the larynx and 119 controls with various nonmalignant diseases were questioned about their exposure to asbestos. All patients were male and age-matched by decades. Of the carcinoma patients, 27.7% had significant exposure to asbestos, as compared to 2.5% of the controls. The maximum age of onset of the carcinoma was a decade less in patients exposed to asbestos than in those with no exposure; i.e., 42% were 51-60 yr old at onset in the exposed group, whereas 48.8% in the nonexposed group were 71-80 yr old at onset. Three types of occupational exposure were recognized: lagging heater equipment, scaling boilers, and unloading raw asbestos. There was no difference in smoking habits between exposed and nonexposed patients, but there was a greater number of smokers in the carcinoma group than in controls. The results indicate an association between asbestos exposure and laryngeal carcinoma; however, they are based on a retrospective study and on a small number of patients.

1263 ADENOCARCINOMA OF THE PANCREAS IN AZASERINE-TREATED RATS. (Eng.) Longnecker, D. S. (Dartmouth Medical Sch., Hanover, N.H.); Curphey, T. J. *Cancer Res.* 35(8):2249-2258; 1975.

Pancreatic adenocarcinoma was studied in azaserine-treated rats. Groups of Wistar rats were given azaserine i.p. at 5 μg/kg once or twice weekly for six months, and autopsied after 6-18 mo. During the first yr, pancreases developed nodules of atypical exocrine cells which seemed to represent hyperplastic foci and encapsulated adenomas. After one yr most pancreases from treated rats were diffusely abnormal and contained many hyperplastic nodules and adenomas, while more than 1/4 had pancreatic adenocarcinoma. No carcinomas or adenomas were observed in control rats. No other organ showed as high an incidence of involvement as pancreas, but renal neoplasms were frequent. Rats were also injected with *O*-(*N*-methyl-*N*-nitroso-β-alanyl)-L-serine i.p. twice weekly at 10 or 90 mg/kg. The tissue distribution of radioactivity following injection of a ^{14}C-labeled sample was similar to that of azaserine; however, this compound is not a direct-acting bacterial mutagen. Rats treated for six months twice weekly i.p. had a higher incidence of nodules of atypical acinar cells than did controls, although the number of nodules per rat was few. No adenomas or carcinomas were found during 13 mo of the study. It is concluded that azaserine

is a carcinogen in rats and causes major abnormalities of growth and differentiation of the exocrine pancreas, including adenocarcinoma in some rats. *O*-(*N*-methyl-*N*-nitroso-β-alanyl)L-serine had less effect that azaserine on pancreatic growth and differentiation.

1264 1-β-D-ARABINOFURANOSYLCYTOSINE-INDUCED MALIGNANT TRANSFORMATION OF HAMSTER AND RAT CELLS IN CULTURE. (Eng.) Kouri, R. E. (Microbiological Associates, Bethesda, Md., 20014); Kurtz, S. A.; Price, P. J.; Benedict, W. F. *Cancer Res.* 35(9):2413-2419; 1975.

The effects of the potent antileukemic chemotherapeutic drug, 1-β-D-arabinofuranosylcytosine, were investigated *in vitro* in hamster fetal cells and rat cells. Exponentially growing hamster fetal cells were collected by trypsinization and added to 60-mm Petri dishes containing 6 x 10^4 lethally irradiated rat cells. After a 24-hr incubation, different concentrations of 1-β-D-arabinofuranosylcytosine-containing medium were added; after a predetermined exposure time, the drug-containing medium was removed, and all plates were refed with medium containing a 10-fold excess of 2'-deoxycytidine relative to the concentration of the drug. The medium was replaced 24 hr later with complete medium and was replaced every 3-4 days until macroscopically visible colonies were observed (10-14 days). The cells were washed; fixed with methanol; stained with May-Grunwald for ten minutes; restained with Giemsa for 20 min; and the number of transformed colonies was determined. Benzo(*a*)pyrene-induced transformation of hamster fetal cells was examined in a similar manner. Exponentially growing rat cells were treated for 24 hr with 10^{-5}M 1-β-D-arabinofuranosylcytosine; the drug was removed, and the cells were incubated an additional 24 hr in a medium containing 10^{-3}M 2'-deoxycytidine. One week after treatment, the cells were split 1:2 and carried serially with weekly transfers by the vertical-horizontal method. Flasks were screened biweekly for areas of transformation. The potential malignancy of the transformed rat and hamster cells was tested by sc inoculation of 1 x 10^6 cells into newborn rats and hamsters, respectively. The results indicate that hamster cells altered by treatment with this drug are morphologically indistinguishable from cells transformed with benzo(*a*)pyrene. The 1-β-D-arabinofuranosylcytosine-induced transformation was seen under conditions of little or no inhibition of DNA synthesis and little or no cytotoxicity. As little as a 6-hr exposure to 1-β-D-arabinofuranosylcytosine was needed for transformation; maximum transformation occurred after 10^{-5} or 10^{-4}M 1-β-D-arabinofuranosylcytosine treatment for 12-24 hr. The transformation of hamster cells seems to require cellular DNA synthesis, because cells in S phase were much more sensitive to 1-β-D-arabinofuranosylcytosine-induced transformation than were G_1 arrested cells. Transformed colonies of hamster and rat cells produced rapidly growing fibrosarcomas in inoculated newborn hamsters and rats, respectively. It is concluded that 1-β-D-arabinofuranosylcytosine can malignantly transform hamster fetal cells and rat cells *in vitro*.

1265 GENETIC DIFFERENCES IN THE AROMATIC HYDROCARBON-INDUCIBLE N-HYDROXYLATION OF 2-ACETYLAMINOFLUORENE AND ACETAMINOPHEN-PRODUCED HEPATOTOXICITY IN MICE. (Eng.) Thorgeirsson, S. S. (Natl. Inst. Child Health Hum. Dev., Bethesda, Md.); Felton, J. S.; Nebert, D. W. *Mol. Pharmacol.* 11(2):159-165; 1975.

Genetically different C57BL/6N (B6) and DBA/2N (D2) inbred mice were given microsomal enzyme inducers 3-methylcholanthrene (MC), β-naphthoflavone (BNF), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and sodium phenobarbital (PB) to study their effect on genetic differences in *N*-hydroxylation of 2-acetylaminofluorene (2-AAF) and acetaminophen. Each mouse was given MC or BNF, 80 mg/kg ip, 48 hr prior to sacrifice. PB-treated mice received the drug ip for three days, and were sacrificed 24 hr after the last injection. TCDD-treated mice were given 100 μg/kg 48 hr prior to assay. Hydroxylase activity in the liver was then determined. The results showed an increase in the *N*-hydroxylation of 2-AFF following MC treatment that appeared to segregate as a single autosomal dominant gene in reciprocal matings between B6 and D2 inbred mice, and that the *N*-hydroxylase induction was always associated with induction of aryl hydrocarbon hydroxylase activity by MC. There was a high correlation between the hydroxylase and *N*-hydroxylase activities in the livers of B6 and B2 mice treated with inducers MC, BNF, TCDD, and PB. There was a striking correlation not only with the presence or absence of induction, but also with its magnitude. Prior treatment with MC potentiated both the incidence and severity of acetaminophen-induced hepatic necrosis in B6 mice, but had little effect on D2 mice. The extent of hepatotoxicity caused by acetaminophen administered ip to these two strains of mice was also highly associated with both aromatic hydrocarbon inducible monooxygenase "activities": aryl hydrocarbon hydroxylase and acetylarylamine *N*-hydroxylase. It is suggested that cytochrome P 450 is involved with the aromatic hydrocarbon-inducible *N*-hydroxylase activity, and that these genetic differences among inbred strains of mice offer a valuable experimental model system for studying the mechanism of hepatoxicity and carcinogenicity among siblings of a defined genotype.

1266 ARYL HYDROCARBON HYDROXYLASE IN LIVER NUCLEI OF C3H/He AND DBA/2 MICE. (Eng.) Watanabe, M. (Res. Inst. for Tuberculosis, Leprosy and Cancer, Tohoku Univ., Hirose-machi 4-12, Sendai 980, Japan); Ariji, F.; Takusagawa, K.; Konno, K. *Gann* 66(4):399-409; 1975.

Microsomal aryl hydrocarbon hydroxylase activity in mice treated with 3-methylcholanthrene or phenobarbital was investigated. Four female (5- to 6-wk-old) C3H/He and DBA/2 mice were given 1 mg 3-methylcholanthrene/10 g body wt intragastrically. Phenobarbital (1 mg/10 g) was administered ip into eight mice of each strain for three days. The mice were sacrificed 24 hr after the last treatment. Aryl hydrocarbon hydroxylase activity in liver nuclei from the C3H/He mice was increased by the administration of 3-methylcholanthrene, but the enzyme activity from the

./2 mice was not. Phenobarbital caused increased ivity in both strains. Approximately the same els of apparent K_m for benzo(*a*)pyrene occurred in er nuclei from both strains of mice even after atment with 3-methylcholanthrene, but different values for NADPH and NADH were observed between constitutive and induced enzyme, showing 0.032 0.091 mM for NADPH and 0.303 and 1.67 mM for H, respectively. Both 5,6-benzoflavone and 7,8-zoflavone (each 10^{-4} M) enhanced the activity in constitutive enzyme, but inhibited it in the luced enzyme noncompetitively. Cyclohexene oxide 1,1,1-trichloro-propane oxide (0.2 mM) enhanced activity in the induced enzyme, but not in the nstitutive enzyme in liver nuclei. The differences the properties between the constitutive and in-ed enzymes, and between the enzymes in microsomes in nuclei from mouse liver are discussed.

57 STUDIES ON RAT COMPLEMENT: II. COMPLEMENT LEVEL IN EXPERIMENTAL TUMOR IN RATS. g.) Sakamoto, M. (Dept. Home Economics, Wayo en's Univ., Konodai, Ichikawa 272, Japan); Nish-a, K. *Jpn. J. Exp. Med.* 45(3):191-198; 1975.

plement (C) levels in male and female rats were sured during the course of carcinogenesis in-ed with methylcholanthrene (MC, 10 mg, sc) or ethylaminoazobenzene (DAB, 0.06%, po), and after infection with *Corynebacterium*. CIA50, C4, and levels increased in MC-treated rats compared h controls, and the increase was correlated with or size greater than 10 g but less than 30 g. comas over 7 g developed in 15 of 23 rats treated h MC. In the case of DAB carcinogenesis, CIA50 C3 levels decreased; however, an elevation of plement level was observed in rats splenecto-ed prior to DAB feeding. Hepatomas had devel-d in all of six animals autopsied after ten ths of DAB feeding and in 6 of 8 splenectomized s autopsied during six months of DAB feeding. 50 and C3 levels increased one week after *Coryne-terium* infection, and rats with increased levels not develop tumors when injected with 3 x 10^6 30 rat ascites hepatoma cells. This cytotoxic ect was not due to the direct action of *Coryne-terium* because injection of *Corynebacterium* si-taneous with, or one day after tumor inoculation not show any antitumor effect. The possibility a cross-reactive antibody with AH130 present in sera seven days after *Corynebacterium* infection also excluded because all rats injected with or cells mixed with postinfection sera died from or growth. Resistance induction against AH130 a more likely explanation because of the increased and CIA50 levels in rats at the time of inocula-on.

68 INVESTIGATIONS OF THE INFLUENCE OF IMMUNODEPRESSIVE MEANS ON THE CHEMICAL CARCINO-NESIS IN RATS. (Eng.) Schmahl, D. (German Cancer s. Center, D-6900 Heidelberg, Kirschnerstrasse 6, st Germany). *Z. Krebsforsch.* 81(3-4):211-215; 74.

e effects of immunodepressants (cyclophosphamide and hydrocortisone) on local carcinogenesis by 3,4-benzopyrene and systemic carcinogenesis by cyclophosphamide were studied in male Sprague-Dawley rats. In the first series of experiments, rats were divided into three groups of 32 animals each and treated with cyclophosphamide alone (13 mg/kg/wk, iv; total dose, 670 mg/kg), hydrocortisone alone (50 mg/kg/wk, sc; total dose, 2600 mg/kg), or cyclophosphamide plus hydrocortisone. In the second series of experiments, rats in groups of 32 received a single injection of 3,4-benzopyrene (3 mg/kg, sc) alone or 3,4-benzopyrene plus hydrocortisone or cyclophosphamide. Treatment with cyclophosphamide alone or with the combination of cyclophosphamide induced malignant tumors in 44% of the animals after latency periods of 510 and 500 days, respectively. Fifteen percent of the animals given hydrocortisone alone showed malignant tumors after 820 days. In the 52 untreated controls, malignant tumors appeared in six animals after 670 days. 3,4-Benzopyrene induced fibrosarcomas at the site of application in 78% of 32 rats after 300 days. The combination of hydrocortisone and 3,4-benzopyrene resulted in malignant tumors in 81% of the animals after 240 days, while the combination of cyclophosphamide and 3,4-benzopyrene induced tumors in 44% of treated rats after 240 days. These results demonstrate that immunodepressive treatment does not promote the carcinogencity of cyclophosphamide and 3,4-benzopyrene. The data also suggest that the carcinogenicity of cyclophosphamide does not depend on its immunodepressive activity but is related to its alkylating effect.

1269 THE EFFECT OF POLYCYCLIC HYDROCARBONS ON THE SYNTHESIS OF DNA IN LYMPHOID ORGANS, BONE MARROW AND REGENERATING RAT LIVER. (Eng.) Prodi, G. (Inst. Cancerology, Univ. Bologna, Via S. Giacomo 14, I-40126, Bologna, Italy); Rocchi, P.; Grilli, S. *Experientia* 31(3):358-360; 1975.

The effects of 7,12-dimethylbenz(*a*)anthracene, 3,4-benzo(*a*)pyrene, dibenz(*a,h*)anthracene (all high oncogenic substances), benz(*a*)anthracene (a weak oncogenic substance), dibenz(*a,c*)anthracene, benzo(*e*)pyrene, and perylene (all nononcogenic substances) were studied in spleen, thymus, femur bone marrow, and regenerating liver of Wistar outbred male rats. Compounds were injected ip in sterile olive oil (0.19 µM/g); ^{3}H-thymidine (18.4 Ci/mmole, 25 µCi/100 g) was injected im 30 min before killing. For spleen, thymus, and bone marrow tests, five animals were killed at 8, 24, 48 hr and 4, 8, and 12 days after injection. For tests on regenerating liver, the hydrocarbons were injected two hours after hepatectomy, and animals (in groups of five) were killed 22 hr later. A constant depression of DNA synthesis was observed, but was not generally correlated with oncogenic power. The maximal effect was exerted by dibenz(*a,c*)anthracene and 7,12-dimethylbenz(*a*)anthracene, the latter having a greater effect on lymphoid organs than on bone marrow. Benzo(*e*)pyrene and benz(*a*)anthracene affected thymus and spleen more than bone marrow. 7,12-Dimethylbenz(*a*)-anthracene and 3,4-benzo(*a*)pyrene exerted a strong inhibition on the DNA labeling in regenerating liver; the benzo(*e*)pyrene was less effective, and the effect of benz(*a*)anthracene was not significant. In the

case of the regenerating rat liver, therefore, there appears to be a correlation between the inhibition of DNA synthesis and the oncogenic power. The lack of a general correlation between oncogenic power and inhibition of DNA synthesis suggests that the depression of DNA synthesis is not a necessary and specific step for the action of chemical carcinogens. The decreased thymidine incorporation probably reflects a toxic action of the compounds used. It is suggested that the inhibition of DNA synthesis may be exerted through weak interactions and intercalations, not correlated to oncogenic power. The inhibition of DNA synthesis, however, could affect the carcinogenic power through an immunosuppressive effect leading to a decreased immunosurveillance.

1270 INHIBITION OF HAMSTER CELL TRANSFORMATION AND OF BENZ(a)PYRENE HYDROXYLATION BY ANTIOXIDANTS. (Eng.) Piekarski, L. (Medical Acad., 02 097 Warszawa, Poland); Konkiewicz, M. *Neoplasma* 22(3):251-253; 1975.

The effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on the benzo(*a*)pyrene (BP)-induced transformation on hamster embryo cells and on BP hydroxylation were determined. The frequency of transformation was calculated as the ratio of transformed colonies to total colonies in cultures treated with 20 nM/ml BP in the presence or absence of the antioxidants. BP hydroxylation was measured as 3-hydroxy-benzo(*a*)pyrene formation in incubation mixtures containing enzyme prepared from secondary cultures of hamster embryo cells after a 24-hr treatment with BP (5 μg/ml) and from liver of rats 18-24 hr after an ip injection of 45 mg/kg BP. Cell transformation was slightly reduced in cells treated with BP and 0.1 μM BHA or BHT, compared with cells treated with BP only. Addition of 0.1 or 1 μM BHA or BHT to the incubation mixture reduced BP hydroxylation.

1271 BINDING OF [^{3}H]BENZO(*a*)PYRENE TO NATURAL AND SYNTHETIC NUCLEIC ACIDS IN A SUBCELLULAR MICROSOMAL SYSTEM. (Eng.) Pietropaolo, C. (Francis Delafield Hosp., New York, N. Y. 10032); Weinstein, I. B. *Cancer Res.* 35(8):2191-2198; 1975.

The mechanism of *in vivo* activation of [^{3}H]benzo(*a*)-pyrene (BP) to a reactive form, and the structure of its nucleic acid adducts were studied. Three to five Holtzman rats were given 20 mg/kg methylcholanthrene or benzo(a)pyrene ip., and killed after 18 hr. A microsomal preparation was made from the liver, and the modified nucleic acids were assayed after isolation by column chromatography. In the presence of rat liver microsomes and reduced nicotinamide adenine dinucleotide phosphate there was covalent attachment of [^{3}H]BP to transfer RNA, DNA, certain synthetic polyribonucleotides, and an RNA species endogenous to the microsomal fraction. Evidence that the binding occurs mainly to guanine and, to a lesser extent, adenine residues and is not simply an artifact of tritium exchange was found. The microsomal-mediated binding of [^{3}H]BP to nucleic acids required NADPH and was inhibited by 7,8-benzoflavone, glutathione, and magnesium. It was enhanced somewhat by the addition of styrene oxide, cyclohexene oxide, and trichloropropylene oxide. These results provide the first evidence that: (1) the microsome-mediated binding of [^{3}H]BP to nucleic acids is not just due to tritium exchange; (2) a derivative of the hydrocarbon is covalently bound to the nucleic acid, and not simply intercalated; (3) there is a preferential binding to guanine residues; and (4) in addition to binding to exogenous nucleic acids, [^{3}H]BP is bound to an RNA species present in the microsomes. The results suggest that nucleic acid binding of this polycyclic hydrocarbon proceeds *via* an epoxide intermediate.

1272 MAGNESIUM OXIDE AS CARRIER DUST IN BENZO[a]PYRENE-INDUCED LUNG CARCINOGENESIS IN SYRIAN HAMSTERS. (Eng.) Stenback, F. (Univ. Nebraska Med. Cent., Omaha); Sellakumar, A.; Shubik, P. *J. Natl. Cancer Inst.* 54(4):861-867; 1975.

The neoplastic progression induced by intratracheal instillation of benzo[*a*]pyrene (BP) and magnesium oxide in male and female Syrian hamsters was compared with that induced by benzo[*a*]pyrene with ferric oxide as carrier dust. The animals were divided into three groups: 1) 48 received benzo[*a*]pyrene and MgO; 2) 48 received BP and ferric oxide; and 3) 90 untreated controls. The average life span was short: 42 wk in group 1, and 36 wk in group 2; the most common cause of death was suffocation due to neoplastic involvement of the respiratory system. At autopsy, the trachea was ligated and the lungs were removed *en bloc* while still fully expanded. Histologic sections were prepared from each lobe of the lungs, larynx, trachea, stem bronchi, and other organs showing gross pathologic changes. BP and MgO produced squamous cell carcinomas and papillomas in the larynx with a latent period as short as nine wk. Most BP + MgO-induced tumors were in the trachea; 26 adenocarcinomas, squamous cell papillomas, or squamous cell-carcinomas were seen, as well as one polyp. A papilloma, squamous cell carcinomas, adenocarcinomas, adenosquamous lesions, and peripheral adenomatoid lesions were seen in the bronchi. BP + MgO rarely caused tumors in other organs; only a few forestomach papillomas, one melanoma on the dorsal skin, and one ovarian carcinoma were seen. Benzo[*a*]pyrene with Fe_2O_3 as the carrier induced a comparable number of histologically similar tumors; however, tumors developed more frequently in the main bronchi. The morphology and location of the tumors apparently depend basically on the carcinogen; the effect of the carrier dust is mainly to increase the number of tumors. Although similar in many respects to Fe_2O_3, MgO is an effective carrier agent and is a valuable alternative to Fe_2O_3 for use in studies on experimental respiratory tumor induction.

1273 K REGION BENZO(α)PYRENE-4,5-OXIDE IS CONJUGATED BY HOMOGENEOUS GLUTHATHIONE S-TRANSFERASES. (Eng.) Nemoto, N. (Natl. Cancer Inst., Bethesda, Md.); Gelboin, H. V.; Habig, W. H.; Ketley, J. N.; Jakoby, W. B. *Nature* 255(5508):512; 1975.

e detoxification of epoxides of polycyclic aromatic drocarbons by conjugation with glutathione (GSH) s studied. Incubation of any one of the pure glu-thione S-transferases from the rat (A,B,C,E, and) or from the human (B and δ) with GSH and the K gion epoxide ^{3}H-benzo(α)pyrene-4,5-oxide always sulted in the formation of the GSH conjugate. The rmation of conjugate was determined from radioac-vity in a single fraction after thin-layer chroma-graphy. Specific activity of the enzymes in con-gating this substrate varied from 4 to 127 nmol/ n/mg.

74 THE INTERACTIONS OF BENZO(a)PYRENE WITH CELL MEMBRANES: UPTAKE INTO CHINESE HAM-ER OVARY (CHO) CELLS AND FLUORESCENCE STUDIES WITH OLATED MEMBRANES. (Eng.) Brunette, D. M. (Medical s. Council Group Periodontal Physiology, 4384 Medi-l Sciences Building, Univ. of Toronto, Toronto, tario, Canada, M5S 1A8); Katz, M. *Chem. Biol. teract.* 11(1):1-14; 1975.

e interactions of benzo(a)pyrene (B(a)P) with the ll surface membrane were studied by measuring ^{3}H-a)P uptake into intact Chinese hamster ovary cells d by studying the interaction of B(a)P with iso-ted cell membranes by fluorescence techniques. was found that 0.19 μg B(a)P were taken up by ^{5} Chinese hamster ovary (CHO) cells after 30 min posure to a solution containing 0.59 μg/ml. Cul-re conditions altered B(a)P uptake markedly. Low ll culture densities resulted in a 4-fold increase rate of uptake per cell relative to confluent nolayer cultures. The uptake was reduced in the esence of bovine serum (BS) and, under some con-tions, perylene. The binding of B(a)P to cell rface membranes could be measured by fluorescence. e methods of data treatment used in the study of uorescent probe-membrane interactions could be plied to get quantitative information on B(a)P-mbrane interactions. It was found that 0.6 x 10^{-8} les B(a)P were bound per mg membrane protein and at the apparent statistical dissociation constant r the complex was 3.8 x 10^{-7} M. The data suggest at the mechanism of uptake of B(a)P is probably ssive diffusion. Its distribution between the dium and cell membrane appears to be influenced physical adsorption as well as by the lipid/water rtition coefficient.

75 THE EFFECT OF DIETARY PHENOBARBITAL ON THE INDUCTION OF SKIN TUMORS IN HAIRLESS CE WITH 7,12-DIMETHYLBENZ[A]ANTHRACENE. (Eng.) ibe, D. D. (Div. Biol. Med. Res., Argonne Natl. ., Ill.); Peraino, C.; Fry, R. J. M. *J. Invest. rmatol.* 64(4):258-262; 1975.

e influence of dietary phenobarbital on 7,12-di-thylbenz[a]anthracene-induced skin carcinogene-s in a population of female, hairless HRS/J/Anl ce was studied. In the first series of experi-nts, mice 24-26 wk old were given either 6 or 12 ekly topical applications of 250 μg of 7,12-di-thylbenz[a]anthracene (DMBA) solution. From the venth week, the mice were placed on either a 30% sein pelleted control or a 0.05% phenobarbital-supplemented diet. In a second series, mice 10-12 wk old were given the first of twelve weekly appli-cations of either 250 or 100 μg of DMBA. The diet was begun two weeks prior to the initiation of treatment. The skin of all animals was examined weekly for 20 wk and bimonthly thereafter. Where treatment and diet were administered concurrently, the incremental appearance of skin tumors during the first week of combined treatment was signifi-cantly reduced; after the cessation of treatment, the incremental appearance of papillomas was com-parable in the two diet groups. Phenobarbital appeared to delay the appearance of papillomas initially, but the suppression of the ultimate tumor yield was variable; the yield appeared to be dependent upon the dose of the carcinogen and on the age of the mice. By the 15th week, all of the younger mice treated with the 250 μg solution bore multiple papillomas and other epidermal tu-mors, and the experiment was terminated. Pheno-barbital was ineffective when DMBA was applied in sufficiently large amounts to elicit marked cutane-ous damage and when diet was begun after the ces-sation of treatment. Although the dietary admini-stration of phenobarbital caused an apparent de-crease in the final incidence of papillomas and sarcomas, it did not appear to modify the macro-scopic skin response or to change the incidence of carcinomas.

1276 SITE OF ORIGIN OF MAMMARY TUMORS INDUCED BY 7,12-DIMETHYLBENZ[a]ANTHRACENE IN THE RAT. (Eng.) Sinha, D. (Dep. Breast Surg., Roswell Park Mem. Inst., Buffalo, N. Y.); Dao, T. L. *J. Natl. Cancer Inst.* 54(4):1007-1009; 1975.

The origin site of mammary tumors after induction by 7,12-dimethylbenz[*a*]anthracene (DMBA) was repor-ted. Sixty-five 55-day-old female Sprague-Dawley rats were used; 31 were given local applications of DMBA (1 mg) in cholesterol "dusted" over surgi-cally exposed glands and 34 were given systemic i.v. injections (3 mg/100 gm). The rats were killed 20, 35, 50 and 85 days after DMBA treatment. Twenty days after either i.v. or local treatment, 42% of the rats in each group had identifiable microscopic neoplastic lesions. Hyperplastic alveolar nodules (HAN) were observed after 35 days, but only in rats given systemic injections of DMBA. At 35 days 55% and 80% of the systemically treated rats and the locally treated, resp., had adenocarcinomas. The tumors all originated in the lumen of the mammary ducts. Tumor formation began as 'piling up' of epithelial cells and progressed into papillary growth filling the lumen. Tumors became palpable much sooner in rats treated by local application of DMBA. These results indicate that tumor induction by DMBA is a direct process, irrespective of the route of administration.

1277 EFFECTS OF 7,12-DIMETHYLBENZ[*a*]ANTHRACENE ON RAT MAMMARY EPITHELIAL CELL MACROMOLE-CULES: A TIME STUDY. (Eng.) Jabara, A. G. (Dep. Pathol., Univ. Melbourne, Australia); Minasian, L. C.; Matthews, J. P. *J. Natl. Cancer Inst.* 55(1): 211-213; 1975.

The effect of 7,12-dimethylbenz[*a*]anthracene (DMBA) on four macromolecules of mammary epithelial cells and on the carcinomas that subsequently developed after Sprague-Dawley virgin female rats were fed DMBA was investigated. The level of nuclear protein changed little during the experiment. Levels of cytosol decreased by 156 μg/mg of nuclear protein and nuclear RNA decreased by 11.5 μg/mg of nuclear protein in mammary epithelium in DMBA-treated rats (24 hr after treatment) compared to normal rats. Both these differences were significant at the < 0.01 level. By the 14th day cytosol had increased by 417.0 μg and nuclear RNA had increased by 16.5 μg/mg nuclear protein. Nuclear DNA was increased 79.5 μg at this time also. Three continuously growing tumors developed in rats fed DMBA and allowed to live for at least 135 days. These tumors were diagnosed histologically as papillary cystadenocarcinoma, adenocarcinoma, and cystadenocarcinoma. The levels of cytosol protein and nuclear RNA in these neoplasms ranged from 5,766 to 6,291 μg/mg of nuclear protein and from 218 to 279 μg/mg nuclear protein, resp. Nuclear DNA level was 678-724 μg/mg of nuclear protein in these neoplasms. The data suggest that early changes in RNA and cytosol protein may be related to DMBA mammary carcinogenesis, and that malignant transformation may occur within the first 4-6 days, after which there appears to be a loss of normal synthetic control of nuclear DNA, RNA, and cytosol protein.

1278 THE EFFECT OF AGING AND INTERVAL BETWEEN PRIMARY AND SECONDARY TREATMENT IN TWO-STAGE CARCINOGENESIS ON MOUSE SKIN. (Eng.) Van Duuren, B. L. (New York Univ. Med. Cent., N.Y.); Sivak, A.; Katz, C.; Seidman, I.; Melchionne, S. *Cancer Res.* 35(3):502-505; 1975.

The effects of age and of the interval between tumor initiation (with 7,12-dimethylbenz(a)anthracene [DMBA]) and promotion (with phorbol myristate) on skin tumor production was studied in female ICR/Ha Swiss mice. Initiation consisted of a single application of DMBA (20 μg in 0.1 ml acetone) and promotion was effected by three applications per week of phorbol myristate (2.5 μg) throughout life. Initiation took place at 6, 44, or 56 wk, with promotion beginning two weeks later. With increasing age, tumors appeared more slowly and the incidence was considerably reduced. Among animals initiated at six weeks with promotion beginning at 2, 36, or 56 weeks, the rate of tumor appearance was somewhat reduced in those with longer intervals between initiation and promotion, although the initiating effect was still present even after an interval of 56 weeks. The shorter life expectancy of mice given carcinogenic treatment later in life is considered to be a factor in the results.

1279 ESTROGEN-PROLACTIN DEPENDENCY IN 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED TUMORS. (Eng.) Leung, B. S. (Univ. Oregon Med. Sch., Portland); Sasaki, G. H.; Leung, J. S. *Cancer Res.* 35 (3):621-627; 1975.

The effects of hormones on 7,12-dimethylbenz(a)anthracene-induced adenocarcinomas in Sprague-Dawley rats were studied by endocrine ablation and hormone replacement. More than 90% of the tumors regressed after bilateral ovariectomy and adrenalectomy, and most of these resumed growth after estrogen therapy (0.1-5 μg/day). About 75% of the stimulated tumors were inhibited by nafoxidine (0.1 mg/day). Prolactin (2 mg on day seven or 11 after ablation) stimulated tumor growth in some animals but not in others, but when given on day two or three after ablation, it stimulated most tumors. Nafoxidine did not inhibit the prolactin-stimulated growth, and withdrawal of both prolactin and nafoxidine was followed by tumor regression. The combination of a low dose of estrogen (0.01 μg) plus prolactin resulted in rapid growth of prolactin-resistant tumors after a lag of about four days, although this level of estrogen alone had no effect on tumor growth. The lag period could be eliminated by priming with estrogen. The results are discussed in terms of the interaction of estrogen and prolactin at tumor sites and the growth regulatory effect of these interactions.

1280 IDENTIFICATION, SUBCELLULAR LOCALIZATION, AND ESTROGEN REGULATION OF PEROXIDASE IN 7,12-DIMETHYLBENZ(a)ANTHRACENE-INDUCED RAT MAMMARY TUMORS. (Eng.) DeSombre, E. R. (Ben May Lab. Cancer Res., Chicago, Ill.); Anderson, W. A.; Kang, Y. H. *Cancer Res.* 35(1):172-179; 1975.

In an effort to find a practical method for distinguishing hormone-dependent from hormone-independent breast cancer, growing, estrogen-dependent, 7,12-dimethylbenz(a)anthracene-induced mammary tumors in Sprague-Dawley rats were examined histochemically. 3,3'-Diaminobenzidine staining prior to electron microscopy revealed an intensely positive reaction for peroxidase in estrogen-dependent tumors, but not in hormone-independent tumors. Activity was localized in the nuclear envelope and cisternae of the granular endoplasmic reticulum as well as compartmentalized in secretory granules. In tumors regressing after ovariectomy, activity was lost, but in such tumors activity was again demonstrated after growth had been restimulated by administration of exogenous estradiol (2.5 μg/day). The simplicity of the peroxidase staining reaction and the fact that it could be done at the same time as routine pathological studies suggest further studies of peroxidase activity in human breast cancer.

1281 FAILURE OF ASCORBIC ACID TO INHIBIT FANFT-INDUCED BLADDER CANCER. (Eng.) Soloway, M. S. (Univ. Hosp., Cleveland, Ohio 44106); Cohen, S. M.; Dekernion, J. B.; Persky, L. *J. Urol.* 113(4): 483-486; 1975.

The effect of concomitant administration of L-ascorbic acid on the resultant incidence of bladder tumors induced by N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) was evaluated in female C_3H/He mice. The mice received either a normal diet, normal diet plus L-ascorbic acid (250 mg/100 cc water), FANFT (0.1% concentration), or FANFT plus L-ascorbic acid. Histological studies were made of bladders after 10 months. All of the mice ingesting FANFT alone (41)

d tumors and 92% (12 of 13) of the mice ingesting NFT with L-ascorbic acid in their drinking water d tumors. There was a wide range in tumor size d depth of invasion within each group, but there s no significant difference between the mice with d without concomitant vitamin C. The tumors were ways transitional cell although squamous metaplasia s present in some instances. It is concluded that corbic acid has no proved effect on bladder tumor rmation and unless evidence indicating otherwise determined, it has no indication as a therapeutic prophylactic agent for human bladder cancer.

32 MUTAGENICITY STUDIES WITH CAPTAN, CAPTOFOL FOLPET AND THALIDOMIDE. (Eng.) Kennedy, L., Jr. (Ind. Bio-Test Lab., Inc., Northbrook, l.); Arnold, D. W.; Keplinger, M. L. *Food Cosmet. ricol.* 13(1):55-61; 1975.

ree fungicides, captan (N-trichloromethylthiotetra-drophthalimide), captofol (N-1,1,2,2,-tetrachloro-hylthiotetrahydrophthalimide), and folpet (N-tri-loromethylthiophthalimide), and the structurally lated compound thalidomide were tested for muta-ic effects in a dominant lethal study using 60-day old Charles River strain albino mice, and in host-mediated assay. In the dominant lethal study le mice were given a single ip injection of captan ptofol, folpet, or thalidomide in doses of 3 or 6 /kg, 1.5 or 3.0 mg/kg, 5 or 10 mg/kg, and 500 or 00 mg/kg, respectively. They were then housed h groups of three virgin females for the next six ks. Each group was removed after one week in the eeding cage. Females were killed by CO_2 asphyxi-on one week after removal, and the number of im-ntation sites were recorded. Preimplantation s, the percentage of deciduomas (early deaths), the percentage of embryos in test *versus* control ales were determined. A histidine-dependent ain of *Salmonella typhimurium* (his G46) was used the host-mediated assay; it was given po in a ubation of captan, captofol, or folpet at 125 or mg/kg, or 500 or 1,000 mg thalidomide/kg. Posi-e and negative control groups were used, the posi-e group receiving an im injection of 100 mg di-hylnitrosamine/kg. Treatment was continued for days, and the animals were killed three hours er the final treatment. Serial dilutions of the itoneal washing were prepared in sterile normal ine and cultured on either histidine-supplemented histidine-deficient agar. After a 48-hr incuba-n, the mutation rate was determined (ratio of totrophs to total organisms). The dominant lethal dy showed a somewhat low pregnancy rate in the up receiving 10 mg folpet/kg, but this was not sidered unusual for this strain. Indices for all er groups compared favorably with those of con-ls. In the host-mediated assay, no increases in spontaneous control rate were obtained in the ber of revertants to prototropic type from the ated rats. Dimethylnitrosamine-treated animals revertant rates some 5-10 times higher than nor-. The results indicated that captan, captofol, folpet are not mutagenic in either of the two t systems.

1283 MUTAGENIC ACTIVITY OF FURYLFURAMIDE ON CULTURED MOUSE CELLS. (Eng.) Umeda, M. (Yokahama City Univ. Sch. Medicine, Urafunecho, Minami-ku, Yokahama 232, Japan); Tsutsui, T.; Kikyo, S.; Saito, M. *Jpn. J. Exp. Med.* 45(3):161-170; 1975.

Effects of furylfuramide (FF) on cultured FM3A cells, a C3H mouse mammary carcinoma cell line, were examined. FF was dissolved in dimethyl sulfoxide and added to the media at 10^{-4} M or $10^{-4.5}$ M. Growth curves were drawn using the replicate tissue culture method. FF inhibited the growth at $10^{4.5}$ M, and provoked enlargement of cellular, nuclear and nucleolar size, cytoplasmic vacuolation and granular aggregation of chromatin. Chromosome preparation demonstrated severe aberrations in nearly 90% of mitotic plates after 24- and 48-hr treatment. The changes included gaps, breaks, exchanges and frag-mentations, but were at chromatid level. Time course study of the incorporation of radioactive precursors showed the gradual but remarkable inhi-bition of ^{3}H-thymidine uptake, whereas ^{3}H-uridine and ^{3}H-leucine uptakes were maintained without sig-nificant inhibition. Thus, it is suggested that FF induced the block of G_1 phase and the delay of S and/or G_2 phase. According to the results of alka-line sucrose gradient analysis of cell DNA, breakage of the treated cell DNA was induced, and the induced breakage was recovered after the incubation without FF. The induced 8-azaguanine (8AG)-resistant mutant cells in FM3A cells by the treatment of test com-pounds were also determined by treating the cells with various concentrations of FF for two days, and then inoculating them on medium with or without 20 μg/ml/8AG. After about 12-14 days of incubation, the capacity to induce 8AG-resistant mutant cells by FF was remarkably high.

1284 CHROMOSOME DAMAGE INDUCED BY GENTAMICIN IN MOUSE L-CELLS. (Eng.) Leonard, A. (Radiobiology Dept., C.E.N.-S.C.K., Boeretang 2000, B-2400 Mol Belgium); Botis, S. *Experientia* 31(3): 341-343; 1975.

The antibiotic gentamicin derived from *Micromonospora purpurea* was studied to determine its effect on tis-sue cultures of mouse L-cells where it is used to prevent mycoplasma contamination. The L-cell strain used was characterized by an average chromosome num-ber of 60, including 17 nonacrocentric chromosomes. Control cells were compared with cultures treated with 500 μg/ml gentamicin for four days followed by two or five passages with 100 μg/ml. TC chromosome arresting solution (1 ml) was added 2.5 hr before termination; cells were fixed, spread on clean slides, and stained. Treatment with 500 μg/ml gentamicin fol-lowed by two passages with 100 μg/ml did not induce changes in the number of L-cell chromosomes. When initial treatment with 500 μg/ml was followed by five passages of 100 μg/ml, cells showing 60 chromosomes dropped to less than 30%. Chromosome fragments and dicentrics were frequent and the mitotic index was significantly decreased in L-cells treated in five passages of 100 μg/ml gentamicin. Since gentamicin induces chromosomal changes, there must be control

over the characteristics of cell strains used experimentally.

1285 DIFFERENCES BETWEEN PURIFIED ECTOPIC AND NORMAL ALPHA SUBUNITS OF HUMAN GLYCOPROTEIN HORMONES. (Eng.) Weintraub, B. D. (Natl. Inst. Arthritis, Metabolism, Digestive Diseases, Bethesda, Md. 20014); Krauth, G.; Rosen, S. W.; Rabson, A. S. *J. Clin. Invest.* 56(4):1043-1052; 1975.

"Ectopic" proteins, immunologically indistinguishable from the common alpha subunits of the glycoprotein hormones, were purified approximately 10,000-fold from a gastric carcinoid tumor (A.L.-α), and from tissue culture medium of bronchogenic carcinoma, cell lines (ChaGo-α). The concentration of alpha subunit was determined by double-antibody radioimmunoassay, and gonadotropin-binding activity was determined by radioreceptor assay using human chorionic gonadotropin (hCG) as a standard. Combination of alpha with beta subunits was tested by incubation of hCG alpha subunits (hCG-α) and ectopic alpha subunits with hCG beta subunits (hCG-β) and measuring the degree of combination by the appearance of activity in the gonadotropin radioreceptor assay. The apparent molecular wt of the various alpha subunits were determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, and by gel chromatography on calibrated Sephadex G-100 columns. Amino acid analyses were performed with a Beckman 120-C two-column analyzer. The purified A.L.-α was homogenous by SDS gel electrophoresis,s, while the apparently less pure ChaGo-α showed multiple components. The apparent molecular wt of A.L.-α was 15,000, significantly lower than the value of 22,000-23,000 for alpha subunits of hCG-α and other glycoprotein hormones. This difference may have resulted from diminished binding of SDS by the normal, compared to the ectopic, alpha subunits. In gel chromatography, however, the molecular wt of A.L.-α was 27,000. The composition of A.L.-α was similar to that of hCG-α in 13 amino acids, but showed decreased phenylalanine and increased valine. A.L.-α combined with hCG-α to produce only 2% of the expected gonadotropin-binding activity, while hCG-α combined with hCG-β to give 95% of the expected activity. It is suggested that these characteristics of ectopic alpha subunits reflect abnormalities of neoplastic protein synthesis. Alternatively, the ectopic subunits may represent as yet unrecognized alpha precursor forms.

1286 MECHANISM OF THE MUTAGENIC ACTION OF HYDROXYLAMINE. VIII. FUNCTIONAL PROPERTIES OF THE MODIFIED ADENOSINE RESIDUES. (Eng.) Budowsky, E. I. (Shemyakin Inst. Bioorganic Chem., USSR Acad. Sci., Moscow); Sverdlov, E. D.; Spasokukotskaya, T. N.; Koudelka, J. *Biochim. Biophys. Acta* 390(1):1-13; 1975.

Inductions of conversions of T4 phage *amber* and *ochre* rII mutants by treatment with *O*-methylhydroxylamine were used as a means of tracing the functional activity and specificity of the 6MeOAdo-type modified adenosine residues. The phages were incubated in 1.0 M *O*-methylhydroxylamine solution containing 0.1 M NaCl and 5 x 10^4 M Versene at pH 5.0, 37C. Aliquots were plated with *Escherichia coli* B and KB to count the revertants, and on *E. coli* CAJ 64 to count the *ochre* to *opal* convertants. There was a considerable increase in the frequency of reversions and conversions of *ochre* and *opal* mutants, probably due to transitions from adenine to guanine. The transitions seemed to be caused by modification of the adenine bases of the genome to give N^6-methoxyadenosine, which is similar to guanine in functional specificity. The functional activity of N^6-methoxyadenosine residues was also studied in the RNA polymerase system, and was found to be low. No significant activity of these residues was detected, either as components of a template polyribonucleotide or as nucleoside triphosphate precursors. The previously reported extensive mutagenesis of intracellular T4 phage and of yeast caused by the addition into the medium of N^6-hydroxyadenine suggests that N^6-hydroxyadenosine residues are functionally active and have a dual functional specificity.

1287 IMPORTANCE OF THE FECAL STREAM ON THE INDUCTION OF COLON TUMORS BY AZOXYMETHANE IN RATS. (Eng.) Campbell, R. L. (Sch. Med., Wayne State Univ., Detroit, Mich.); Singh, D. V.; Nigro, N. D. *Cancer Res.* 35(5):1369-1371; 1975.

The importance of the presence and composition of the fecal stream in intestinal carcinogenesis produced by azoxymethane was studied in 100 male Sprague-Dawley rats. Colostomies were performed on 50 animals at a point approximately 2 cm distal to the cecum; the other rats were kept intact. In each of these groups, half the rats were fed a normal diet and half were fed a diet containing 2% cholestryamine. All rats were given weekly sc injections of azoxymethane (8 mg/kg). After seven mo all surviving animals were sacrificed, and the distal 20 cm of colon was removed. Nine tumors, each 0.5 cm in greatest diameter, were taken from each group. All of these were identified histologically as adenocarcinomas. Several rats died from pneumonia early in the study, leaving 15 rats in the colostomy-normal diet group and 22 in each of the other groups. The average number of tumors per rat was 2.3 for the colostomy-normal diet group, and 4.9 for intact rats fed a normal diet. In the groups fed cholestyramine, those with defunctionalized colons had an average of 2.6 tumors per rat while intact rats had 6.2 tumors per rat. These results support the epidemiological evidence relating diet and fecal composition to the incidence of colon cancer. The data indicate that the effect of azoxymethane on the colon is influenced by the fecal stream and that the carcinogens can also affect the colon by a route other than the fecal stream.

1288 NICKEL CHLORIDE-INDUCED METABOLIC CHANGES IN THE RAT AND GUINEA PIG. (Eng.) Clary, J. J. (Natl. Inst. Occup. Saf. Health, Cincinnati, Ohio). *Toxicol. Appl. Pharmacol.* 31(1):55-65; 1975.

The toxic effects of nickel chloride on body metabolism were investigated with regard to the mechanism of action involved. Nickel chloride was administered to male albino rats by various routes (intratracheal, 0.2 ml, 5 mg ^{63}Ni/ml, 10 μCi/ml; ip, 8 mg Ni/kg; four months in drinking water, 225 ppm) and

male albino guinea pigs (sc, 1 mg $^{63}Ni/kg$, 2 :i/day). In addition, and intragastric [^{14}C]glu-
se load (600 mg) was also given to 20 intratrache-
ly injected rats. The following parameters were
asured in the serum to assess the effect of Ni on
tabolism: total ^{14}C radioactivity, glucose, in-
lin, total lipids, cholesterol, and triglycerides.
ver glycogen and glucose-6-phosphatase activity
s measured to determine glucose turnover in the
ver. ^{63}Ni tissue distribution and excretion of
Ni, calcium, sodium, potassium, zinc, and glucose
re also measured as a determination of metal and
rbohydrate metabolism. A single ip or intratrache-
injection of Ni (0.5 mg) to rats caused a rapid
ansient increase in serum glucose, but a decrease
serum insulin and glucosuria. When exogenous
sulin (0.5 U or 0.25 U, im) was given at the same
me as the nickel challenge, the elevation of serum
ucose was prevented. Glucose turnover studies
dicated that the mechanism of action of nickel
pears to be in the inhibition of insulin release.
is inhibition of insulin release could be related
the extremely high concentration of nickel found
the pituitary and the effect of nickel on the
cretion of the pituitary hormones (growth hormone
d ACTH).

89 DISAPPEARANCE OF NITRITE FROM THE RAT STOMACH: CONTRIBUTION OF EMPTYING AND HER FACTORS. (Eng.) Mirvish, S. S. (Univ. Ne-
aska Med. Cent., Omaha, Neb.); Patil, K.; Ghad-
ian, P.; Kommineni, V. R. C. *J. Natl. Cancer Inst.* (4):869-875; 1975.

trite disappearance from the rat stomach was mea-
red after food containing nitrite was given in or-
: to study how intragastric nitrosation forms N-
troso compounds. The *in vitro* disappearance of
trite from buffered aqueous solutions and food
irries was measured in preliminary experiments in
ich the pH was lowered from 5 to 1. In preliminary
periments to determine if nitrite reacts specifi-
lly with gastric juice contents, 1.0 mg $NaNO_2$ was
cubated alone or with 2 mg albumin, 2 mg pepsin,
ng mucin, or 1.0 ml rat gastric contents. Nitrite
covery was 71% without additives, 74-78% in the
esence of the proteins, and 65% in the presence of
gastric contents. Starved MRC Wistar rats were
ven 5 g food containing 1.82 mg $NaNO_3$/g. Nitrate
s not reduced to nitrite in the stomach. Five
ms of foods containing 154 μg $NaNO_2$/g was simi-
:ly administered, and the total stomach and gland-
ir and nonglandular parts were analyzed after 1-5
Weight and nitrite concentration of the stomach
ntents dropped linearly, and the amount of nitrite
opped exponentially (with a half-life of 1.4 hr).
n nitrite concentration in the glandular stomach
μg/g) was less than half that in the nonglandular
mach (43 μg/g). In other experiments, 50 g of
d was mixed with 5 ml of a solution of 6 mg phenol
/ml H_2O; 5 g was fed as before to each rat. These
eriments showed that emptying accounted for 60% of
nitrite loss from the total stomach. The dilu-
n factors (the reciprocals of the concentration
ios) were used to estimate the relative contri-
ion of dilution to the decrease in nitrite con-
tration. From food to the nonglandular stomach, the nitrite concentration decreased 2.1 times due to dilution and 1.7 times due to other causes. From food to the glandular stomach, the nitrite concentration decreased 2.9 times due to dilution, and 3.0 times due to other causes. A comparison of nitrite loss *in vivo* and *in vitro* suggests that two factors are important in the disappearance of nitrite from the rat stomach: (a) decomposition of nitrous acid to give nitric acid and nitric oxide, and (b) reactions of nitrous acid with food components. Despite interspecies differences, the results suggest that most of the ingested nitrite will disappear from the human stomach, but that some will persist and be available for N-nitrosation.

1290 DIETARY MODIFICATIONS AFFECTING THE MUTAGENICITY OF *N*-NITROSO COMPOUNDS IN THE HOST-MEDIATED ASSAY. (Eng.) Zeiger, E. (Div. of Toxicology, Food and Drug Admin., Washington, D. C. 20204). *Cancer Res.* 35(7):1813-1818; 1975.

The effects of various diets (chow, complete semisynthetic, protein-free, all-casein, and 24 hr fast) were studied on the mutagenicity of dimethylnitrosamine (DMNA), *N*-nitrosomorpholine (NM), and *N*-methyl-*N*-nitrosourea (NMU) for *Salmonella typhimurium his* G-46 in the host-mediated assay. Swiss albino mice on the various diets were injected ip with a suspension of the bacteria, followed one hour later by an im injection of the mutagen. The mice were killed at intervals of 5, 10, 15, 20, 30, 60, and 120 min; the peritoneal cavity was opened, and the exudate was withdrawn and plated on agar. NMU was the strongest mutagen, followed by DMNA and NM. The mutagenicity of DMNA and NM, which require metabolic activation for their biological activity, was depressed by the complete semisynthetic diet, as compared to the mutagenicity in mice fed the chow diet. DMNA mutagenicity was depressed by the protein-free diet and enhanced by pure casein, as compared with the complete semisynthetic diet. NM mutagenicity was enhanced by starvation, but results with mice fed the protein-free and all-casein diets were ambiguous. NMU, which does not require metabolic activation for its biological activities, responded in an opposite manner to that of DMNA; its mutagenicity was enhanced by the complete semisynthetic and protein-free diets, but was depressed by the all-casein diet. The results suggest that the ultimate mutagenic derivative of DMNA parallels the formation of its ultimate carcinogenic and hepatotoxic derivatives, leading to the conclusion that the mutagenicity, carcinogenicity, and hepatotoxicity of the compound may be responses to the same metabolite.

1291 CARCINOGEN CHEMISTRY. I. REACTIONS OF PROTONATED DIALKYLNITROSAMINES LEADING TO ALKYLATING AND AMINOALKYLATING AGENTS OF POTENTIAL METABOLIC SIGNIFICANCE. (Eng.) Olah, G. A. (Dep. Chem., Case Western Reserve Univ., Cleveland, Ohio); Donovan, D. J.; Keefer, L. K. *J. Natl. Cancer Inst.* 54(2):465-472; 1975.

The protolytic behavior of seven nitrosamines was

studied by following the time dependence of their nuclear magnetic resonance (NMR) spectra in superacid solutions. Cleavage reactions were followed with solutions prepared by cooling 1 ml of the desired acid and treating it with 0.2-0.3 ml nitrosamine; after the resulting solution was warmed to room temperature the NMR spectrum was determined. The solutions were then heated to effect the cleavage reactions (80-140 C), and spectra were periodically recorded until no further changes took place (five minutes to several days). Three distinct modes of protolytic dialkylnitrosamine fragmentation were observed: (a) In equimolar HSO_3F:SbF_5 ("magic acid"), dimethylnitrosamine was cleaved to the protonated Schiff base of formaldehyde and methylamine, and diethylnitrosamine was similarly converted to the protonated acetaldehyde-ethylamine Schiff base. (b) By contrast, of the five dipropyl- and dibutylnitrosamines studied, all cleaved non-oxidatively under these conditions (with loss of nitrogen gas) to the corresponding propyl or butyl cations. The carbocations thus produced underwent condensation and fragmentation to form the *tert*-butyl cation as the principle product ultimately observable by NMR. (c) The third fragmentation mechanism which involved denitrosation to the dialklammonium ion was observed only as a minor pathway in the sulfuric acid or fluorosulfuric acid protolysis of dimethylnitrosamine. The mechanisms that are postulated for these cleavage reactions, if functioning *in vivo*, could account for several metabolic observations that have proved difficult to reconcile with previous conceptions of nitrosamine metabolism.

1292 HISTOLOGIC AND ELECTRON MICROSCOPY OBSERVATIONS ON DIETHYLNITROSAMINE-INDUCED HEPATOMAS IN SMALL AQUARIUM FISH (*ORYZIAS LATIPES*). (Eng.) Ishikawa, T. (Cancer Inst., Kami-Ikebukuro 1-37-1, Toshima-ku, Tokyo 170, Japan); Shimamine, T.; Takayama*, S. *J. Natl. Cancer Inst.* 55(4):909-916; 1975.

Liver neoplasms were induced in medakas *(Oryzias latipes)* by the addition of diethylnitrosamine (DENA) to their aquarium water at levels of 15-135 ppm for eight weeks. After 13 wk, 21 of 32 fish had developed hepatomas. Histologic type differed in the lesions of different fish and also within individual tumors. Of the 20 hepatomas in which complete serial examination was possible, six were differentiated (i.e., liver cell adenomas and trabecular cell hepatomas); four were poorly differentiated (i.e., with spindle cells, anaplastic polyhedral cells, and a "sarcomatous" pattern); and three were a combination of these two and cholangiolar hepatomas. Electron microscopy revealed an extensive rough-surfaced endoplasmic reticulum in a lamellar pattern, many mitochondria, and several round lysosomes in tumor cells. A few fat droplets with occasional crystalline ghosts were sometimes in the cytoplasm. The Golgi apparatus was not conspicuous. Some cells had highly developed microvilli that showed differentiation toward structures resembling bile capillaries. Medakas are useful for further studies because they are highly susceptible to the carcinogenic effect of DENA, and the time for tumor induction is relatively short.

1293 THE INHIBITION OF DIMETHYLNITROSAMINE-INDUCED RENAL TUMORIGENESIS IN NZO/B1 MICE BY ORCHIECTOMY. (Eng.) Noronha, R. F. X. (St. Louis Univ. Sch. Medicine, St. Louis, Mo. 63104). *Invest. Urol.* 13(2):136-141; 1975.

The oncogenic response of inbred NZO/B1 male and female mice to a single ip injection of dimethylnitrosamine (DMN, 7.15 or 15 mg/kg) was studied together with the effect of gonadectomy on natural and DMN-induced tumors in these mice. The animals were weighed weekly for the first six months and every two weeks thereafter. Records of weight, life span, and pathologic data were analyzed by computer. DMN treatment resulted in significant increases in the incidence and multiplicites of lung tumors in mice of both sexes and of kidney tumors in males. The 7.5 mg/kg DMN dose raised the incidence of kidney tumors in males from 0.3% (1/368 animals) to 33% (7/21). No kidney tumors were induced in females. The incidence of lung tumors increased from 19% in untreated males to 91% in DMN-treated males and from 25% (79/319) in untreated females to 75% (16/21) in DMN-treated females. Doubling the DMN dose to 15 mg/kg raised the incidence of kidney tumors in males to 56% (10/18) and doubled tumor multiplicity from 2.0 to 4.5 per tumor-bearing animal. Female mice did not develop tumors even at this doubled dose of DMN. At the higher dose. lung tumors were seen in 89% of the males and in 100% of the females. A characteristic effect of gonadectomy was the development of cortical adenoma and carcinomas in 55% of males and 42% of females. In males orchiectomy completely inhibited the induction of kidney tumors by DMN (7.5 mg/mg); the kidney tumor incidence was reduced to zero. However, gonadectomy did increase the incidence of lung tumors in untreated and DMN-treated mice of both sexes. Histologically, the renal tumors induced by DMN were adenomas or carcinomas. That orchiectomy before DMN injection abolished the kidney tumor response in males implies that androgens are essential for renal carcinogenesis by DMN in the NZO mouse.

1294 REPLICATION OF HEPATIC DNA IN RATS TREATED WITH DIMETHYLNITROSAMINE. (Eng.) Rajalakshmi, S. (Temple Univ. Sch. Medicine, Philadelphia, Pa. 19140); Sarma, D. S. R. *Chem. Biol. Interact.* 11(4):245-252; 1975.

Replication of DNA containing unrepaired lesions such as depurinated sites, single-strand breaks or methylated bases such *O*-6 and *N*-7 methylguanine was studied in rat liver. Liver DNA was damaged by administering 10 µg dimethylnitrosamine (DMN)/g ip four hours prior to partial hepatectomy in male Wistar rats. The analysis of DNA on an alkaline sucrose gradient revealed considerable damage to the parental strand at the time of and 48 hr subsequent to the hepatectomy. During this time interval, the synthesis of new strands was studied using labeled thymidine. In the control liver, radioactivity in DNA appeared as small fragments at 15 and 30 min following the administration of labeled thymidine that became bigger within four hours. In the carcinogen-treated livers, the newly made DNA remained as small

agments for longer periods of time. Sometime between 4 and 24 hr these became larger in size than the rental damaged template DNA. Thus, with a delay, e newly made strands eventually enlarged, in spite the fact that the parental template DNA strand s damaged. Such replication of DNA with unrepaired sions (miscoding and/or noncoding) offers a mechasm by which the original damage to DNA caused by e carcinogen can be permanently imprinted on the wly made cell, a phenomenon that could account for e initiation of carcinogenesis under certain cirmstances.

)5 TRANSFORMATION OF HUMAN CELLS IN CULTURE BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE. ng.) Rhim, J. S. (Microbiol. Assoc., Bethesda,); Park, D. K.; Arnstein, P.; Heubner, R. J.; sburger, E. K.; Nelson-Rees, W. A. *Nature* 256 520):751-753; 1975.

e transformation of human osteosarcoma clonal cells *vitro* by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and e production of tumors by these transformed cells en injected into NIH nude athymic mice are repor-. A human osteosarcoma clonal cell line (grown l maintained in Eagle's minimal essential medium h 10% fetal bovine serum, 2 mM glutamine, and ibiotics) was used for the experiments. One day er plating 2×10^5 cells/ml from the 31st subture, the medium was removed and replaced with ia containing *N*-methyl-*N'*-nitro-*N*-nitrosoguanie at various concentrations (5.0 μg/ml-0.01 μg/ in 0.5% dimethyl sulfoxide. After seven days of atment with the carcinogen, the cultures were hed, fed again with carcinogen-free growth mem, and subsequently passaged by trypsin treatment ry seventh day. Morphological alterations of ls and abnormal growth patterns were noted in the th subculture, 55-59 days after treatment. Doses 1.0 μg carcinogen/ml were lethal. An increased wth rate (double that of the controls) was obved in the *N*-methyl-*N'*-nitro-*N*-nitrosoguanidineated cells. When inoculated sc into nude athymic e, 0.01 μg/ml of the carcinogen-treated cells proed subcutaneous tumors, and 0.1 μg/ml produced ll persistent tumor nodules. The tumors were gressive and transplantable. These results destrate the transformation *in vitro* by *N*-methylitro-*N*-nitrosoguanidine of human cells from a tinuous cell line.

5 TRANSPLACENTAL INDUCTION OF PRIMARY RENAL TUMORS IN RABBITS TREATED WITH 1-ETHYL-1 ROSOUREA. (Eng.) Fox, R. R. (Jackson Lab., Bar oor, Maine, 04609); Diwan, B. A.; Meier, H. *J. l. Cancer Inst.* 54(6):1439-1448; 1975.

nsplacental induction of primary renal tumors by thyl-nitrosourea (ENU) in the offspring of strain and strain WH rabbits was studied. ENU was disved in trioctanoin (17.5 mg/ml), and a dose of 60 g was administered ip to pregnant rabbits on the n day of gestation. Controls received an equivat volume of solvent. Latency time was designated time from birth to death or until the time of the sence of palpable tumor. Rabbits were killed either when moribund or when tumors were palpable. Of the 24 strain III and 14 WH offspring, 16 died of unrelated causes. Of the 15 strain III surviving offspring, 14 developed primary renal tumors, and mean latency was 3.3 mo. Of the seven remaining WH offspring, three had primary renal tumors. Histologically, two tumor types were identified: papillary cystadenoma, and a Wilms'-like nephroblastoma. In both strains, each of these types arose in renal cysts. Five 4-day-old strain III rabbits, pre-treated with goat antirabbit lymphocytic serum (GARLS) received sc injections of renal tubular cystadenocarcinoma cells; GARLS was given twice weekly for five weeks after tumor transplantation. One of the three survivors that had not died of other causes developed three tumors at one year of age, and these were of the transplanted type. It is concluded that primary renal tumors can be readily transplacentally induced, with a single dose of ENU in both strains. The higher incidence in strain III is suggested by the author to be due to the genetic frequency of renal cysts. This strain is a suitable model for studying the two related types of induced renal tumors.

1297 STUDIES ON RECOVERY FROM CHEMICALLY INDUCED DAMAGE IN MAMMALIAN CELLS. (Eng.) Barranco, S. C. (Univ. Texas Med. Branch, Galveston); Novak, J. K.; Humphrey, R. M. *Cancer Res.* 35(5): 1194-1204; 1975.

Survival responses of Chinese hamster ovary cells to drug-induced potentially lethal damage (PLD), and to sublethal damage (SLD) were studied in dividing and nondividing populations. Survival was determined by the ability of the cells to form colonies of 50 or more cells. The greatest difference in the fraction surviving followed treatment with 10 μg/ml 1-trans-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea for one hour; the fraction surviving in the dividing population was about 100 times that of the nondividing population. Treatment with 10 μg/ml of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea gave a survival fraction in dividing cells five times that of nondividing cells. Cells treated with either compound failed to recover from SLD or PLD. With bleomycin (100 μg/ml), survival fraction of nondividing cells was 0.015; of dividing cells, this fraction was 0.14 when cells were plated for colony-formation immediately. If cells were held in depleted (plateau-inducing) medium for 2 or 4 hr before plating, survival fraction was 4-5 times greater in dividing populations than in nondividing populations. These drugs were prepared in balanced salt solution to eliminate serum-drug interactions. The study of recovery of cells from drug treatment may have clinical significance because surviving tumor cells can contribute to the regrowth of the tumor.

1298 PHENACETIN ABUSE AND TUMORS OF THE URINARY TRACT. (Ger.) Landmann-Kolbert, C. (Urolog. Klinik, Chirurg. Departement d. Universität Basel CH-4004 Basel Kantonspital Schweiz); Rutishauser, G.; Dubach, U. C. *Urologe* [*A*] 14(2):75-79; 1975.

Sixty-nine patients with epithelial tumors of the

urinary tract were investigated for abuse of phenacetin-containing analgesics. Abuse of phenacetin-containing analgesics (ranging from 6 tablets/day to 1/wk for 2-20 yr) was demonstrated in 12 tumor patients (17.4%), including 5 men and 7 women. This group included eight cases of papilloma of the bladder, three of carcinoma of the bladder, and one of carcinoma of the renal pelvis and urethra. An increased level of *N*-acetyl-p-amino-phenol, a metabolite of phenacetin, was found in the urine in 6 of these 12 patients. In a control group of mixed nonurological tumor patients of similar sex and age distribution, only 5 pt (7.2%) were found to be phenacetin abusers. Although the difference is not significant statistically, the findings suggest causal connection between phenacetin abuse and tumors of the urinary tract.

1299 EFFECT OF PLUMBOUS ION ON MESSENGER RNA. (Eng.) Farkas, W. R. (Univ. Tennessee Memorial Res. Center, Knoxville, Tenn. 37920). *Chem. Biol. Interact.* 11(4):253-263; 1975.

The effects of Pb^{2+}, a potent catalyst for the depolymerization of RNA were studied on brome mosaic virus (BMV) RNA, rabbit globin messenger RNA (mRNA) and polyuridylic acid. After exposure of these natural and synthetic messengers to a sufficiently high concentration of lead acetate, they all lost their ability to stimulate amino acid incorporation in cell-free protein-synthesizing systems. There were differences in the susceptibilities of the messengers; globin mRNA being the least stable. Varying the Pb^{2+} concentration and exposing the mRNA (1.98 μg) for 40 min revealed that there was a threshold Pb^{2+} concentration below which no loss of mRNA activity was observed. The threshold concentration (0.10 mM) was considerably greater than the Pb^{2+} concentration at which protein synthesis was inhibited in reticulocytes, and overt symptoms of plumbism were observed. However, when mRNAs were incubated for an extended period (24 hr), even with subthreshold concentrations of Pb^{2+}, there was destruction of messenger function, and globin mRNA was more susceptible than BMV-RNA. Also, the susceptibility of mRNA to Pb^{2+} was temperature-dependent. (At 15 C, 20 C, and 25 C, only 24%, 36%, and 76% of the mRNA activity was lost, respectively; total mRNA degradation took place only when the temperature was increased to 30 C.) This indicates that mRNA, like transfer RNA, exists as a population of molecules in different conformational states that are not readily interconvertible.

1300 RESERPINE AND CHEMICAL CARCINOGENESIS. (Eng.) Marquardt, H. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.). *Lancet* 1(7912): 925-926; 1975.

The capacity of reserpine to induce malignant transformation of cells in culture was investigated. A line (M2) of mouse fibroblasts which was previously found to be susceptible to malignant transformation by various chemical carcinogens was used. The percentage of survivors relative to the solvent control are as follows: 10.0 μg/ml, 22% survivors; 5.0 μg/ml, 47% survivors; 2.5 μg/ml, 67% survivors; 1.2 μg/ml, 85% survivors; 0.6 μg/ml, 100% survivors; and 0.3 μg/ml, 100% survivors. Reserpine thus failed to induce malignant transformation *in vitro*.

1301 QUANTITATION OF TRYPTOPHAN METABOLITES IN RAT FECES BY THIN-LAYER CHROMATOGRAPHY. (Eng.) Anderson, G. M. (Natl. Cancer Inst., Frederick, Md.) *J. Chromatogr.* 105(2):323-328; 1975.

Thin-layer chromatographic methods are described for quantitating fecal tryptophan metabolites, which may have an etiologic role in colonic cancer. Indole, skatole, indole-3-acetic acid, indole-3-propionic acid, tryptamine, and free tryptophan were processed by a method involving an initial ethanol homogenization of feces followed by liquid-liquid extraction and thin-layer chromatographic quantitation. Other tryptophan metabolites (indole-3-lactic acid, indole-3-acrylic acid, anthranilic acid indole-3-acetamide, tryptophol, and kynurenine) were recovered in yields of 87-97% from supplemented fecal samples. Detection limits for all compounds studied were in the range of 0.1 μg/g feces. Accuracy of quantitation in the range of μg/g is estimated at ± 10%.

1302 FURTHER RESULTS IN POLYVINYL CHLORIDE PRODUCTION WORKERS. (Eng.) Lange, C. E. (Dept. Dermatol., Univ. Bonn, West Germany); Juhe, S.; Stein, G.; Veltman, G. *Ann. N.Y. Acad. Sci.* 246:18-21; 1975.

The possible nature and extent of occupational damage in 15 employees of polyvinyl chloride (PVC)-processing industries was investigated. In addition, case studies of two former workers of a PVC-producing plant, who had died of malignant tumors, are presented. Dermatological examinations and the following laboratory tests were performed on all 15 workers: complete blood cell platelet count, RBC sedimentation rate, serum electrophoresis, blood urea nitrogen, and Australia antigen. Employed for an average of five years, the workers had complained of sensations of pressure and/or pain in the upper abdomen, frequent dizziness, cold hands and feet, and increasing weakness in the legs. Clinical changes of Raynaud's syndrome were not observed. Slight to moderate thrombocytopenia (63,000-139,000/μl) was observed in seven patients; increased bromosulfthalein retention of 5.2-15.1% at 45 min was noted in another seven patients. Reticulosis of 1.7-4.4% was seen in six patients. Leukopenia (3250/μl) was seen in one patient, and slight splenomegaly was observed in another patient. Laparoscopy and biopsy results were less distinct, though similar to those observed in PVC-production workers. Despite the small number of workers examined, an increased number of findings were made of a combination of symptoms believed characteristic of vinyl chloride disease. A 38-yr-old man, employed for 12 yr, had a large tumor assigned to the liver and spleen, increased ESR, anemia, and other abnormal laboratory findings; surgical laparatomy and biopsies revealed a hemangioendothelial sarcoma of the liver, and increasing cachexia resulted in death. A 39-yr-old man, employed for 11 yr, had

tumor on the epigastric angle, abnormal laboratory esults, a histological diagnosis of hemangioendothelal liver sarcoma, and resulting death.

303 VINYL CHLORIDE EXPOSURE AND HUMAN CHROMOSOME ABERRATIONS. (Eng.) Ducatman, A. Mt. Sinai Sch. Med. City Univ. New York, N. Y.); irschhorn, K.; Selikoff, I. J. *Mutat. Res.* 31(3): 63-168; 1975.

he lymphocyte chromosomes of 11 male subjects reeatedly exposed to vinyl chloride were examined or aberrations. Controls consisted of six men outide the factory environment and four men from within the same factory but without exposure. The averge age of controls was 27, of the subjects 40. uration of vinyl chloride exposure ranged from 4-3 yr with a mean of 15 yr. Ambient gas levels were ssumed to exceed 500 ppm at times. Gaps and breaks ere the predominant aberrations among the 1,050 ells studied. However, exposed subjects had staistically nonsignificant increases when compared o controls. Differences in cells with stable aberrations and with random chromosome loss were also onsignificant. However, cells with unstable aberations were observed in significantly more subjects xposed to vinyl chloride. There was a nonsignifiant increase in total simple breaks of multiaberant cells in exposed subjects. Complex breaking oints were significantly higher in exposed subjects. xaggerated secondary constrictions of the No. 9 hromosome were noted; the average subject had 3.91 hile the average control had 2.00. Despite the mall sample size, it is concluded that excess vinyl hloride exposure is clastogenic.

04 EFFECT OF AFLATOXIN ON PHAGOCYTOSIS OF *ASPERGILLUS FUMIGATUS* SPORES BY RABBIT VEOLAR MACROPHAGES. (Eng.) Richard, J. L. atl. Animal Disease Center, Ames, Iowa 50010); urston, J. R. *Appl. Microbiol.* 30(1):44-47; 75.

05 AFLATOXIN B_1 INDUCED LIVER TUMORS IN *TUPAIA GLIS* (TREE SHREWS) A NONHUMAN IMATE [abstract]. (Eng.) Reddy, J. K. (Univ. nsas Med. Cent., Kansas City); Svoboda, D. J. *d. Proc.* 34(3):827; 1975.

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07 EFFECT OF AFLATOXIN PREINCUBATION AND ADDITION TO RAT LIVER MICROSOMES ON (ED FUNCTION OXIDASE ACTIVITY [abstract]. (Eng.) irclough, D. L. (Virginia Polytech. Inst. State v., Blacksburg); Fox, J. P.; Campbell, T. C. *l. Proc.* 34(3):784; 1975.

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1312 INDUCTION OF ESTHESIONEUROEPITHELIOMAS IN RATS EXPOSED TO BIS(CHLOROMETHYL)ETHER [abstract]. (Eng.) Kociba, R. J. (Health Environ. Res., Dow Chem. Co., Midland, Mich.); Leong, B. K. J.; Jersey, G. C.; Quast, J. F.; Gehring, P. J. *Lab. Invest.* 32(3):428; 1975.

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1323 CHANGES IN THE ACTIVITY OF CHEMICAL MUTAGENS CAUSED BY VARIOUS COMPOUNDS [abstract]. (Eng.) Sram, R. J. (Inst. Hyg. Epidemiol., Prague, Czechoslovakia). *Mutat. Res.* 29(2):195; 1975.

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1352 EXPERIMENTAL MUTAGENESIS IN UNICELLULAR GREEN ALGAE. IV. COMPARATIVE INVESTIGATION OF THE MUTAGENIC EFFECT ON MEMBERS OF THE HOMOLOGOUS SERIES OF N-NITROSO NITROALKYL GUANIDINES IN SCENEDESMUS ACUTUS. (Rus.) Nicolov, N. N. (Inst. Industrial Microbiology, Bulgarian Acad. Sciences, Sofia, Bulgaria); Miladenova, L. N. *Genetika* 11(2):73-78; 1975.

1353 INCREASED CARCINOGENICITY OF 2,6-DIMETHYLNITROSOMORPHOLINE COMPARED WITH NITROSOMORPHOLINE IN RATS. (Eng.) Lijinsky, W. (Oak Ridge Natl. Lab., Oak Ridge, Tenn.); Taylor, H. W. *Cancer Res.* 35(8):2123-2125; 1975.

1354 ESOPHAGEAL CARCINOGENESIS WITH N-NITROSONORNICOTINE AND DINITROSOPIPERAZINE, MODIFIED BY VITAMIN A OR ENZYME INDUCERS [abstract]. (Eng.) Raineri, R. (Am. Health Found., New York, N.Y.); Hecht, S.; Maronpot, R.; Weisburger, J. H. *Fed. Proc.* 34(3):811; 1975.

1355 PANCREATIC ADENOCARCINOMA IN INBRED GUINEA PIGS INDUCED BY *N*-METHYL-*N*-NITROSOUREA. (Eng.) Reddy, J. K. (Coll. of Health Sciences and Hosp., Kansas City, Kans. 66103); Rao, M. S. *Cancer Res.* 35(8):2269-2277; 1975.

1356 SUSCEPTIBILITY OF UROTHELIUM TO NEOPLASTIC CELLULAR IMPLANTATION. (Eng.) Weldon, T. E. (Univ. Hosp., Cleveland, Ohio); Soloway, M. S. *Urology* 5(6):824-827; 1975.

1357 SENSITIVITY AND MUTABILITY OF DIFFERENT TYPES OF COMMON WHEAT MUTANTS UNDER REPEATED TREATMENT WITH MUTAGENS. (Rus.) Morozova, I. S. (Inst. of Chemical Physics, Acad. of Sciences of the U.S.S.R., Moscow, U.S.S.R.); Zoz, N. N.; Babaiev, M. S. *Genetika* 11(2):24-28; 1975.

1358 *IN VIVO* MUTAGENIC INTERACTION OF NITRITE AND ETHYLENETHIOUREA. (Eng.) Seiler, J. P. (Swiss Fed. Res. Stn., Wädenswil, Switzerland). *Experientia* 31(2):214-215; 1975.

1359 PHENACETIN: A CARCINOGEN FOR THE URINARY TRACT? (Eng.) Rathert, P. (Medical Faculty, Rheinisch-Westfalische Technische, Hochschule, Aachen, West Germany); Melchior, H.; Lutzeyer, W. *J. Urol.* 113(5):653-657; 1975.

1360 A COMPARATIVE ANALYSIS OF A TUMOR PROMOTOR (PHORBOL MYRISTATE ACETATE) AND PHYTOHEMAGGLUTININ ACTION ON HUMAN LYMPHOCYTES [abstract]. (Eng.) Drazich, B. F. (Univ. Minnesota, Minneapolis); Estensen, R. D. *Fed. Proc.* 34(3):842; 1975.

1361 MUTAGENIC EFFECT OF THIO-TEPA IN LABORATORY MICE. V. THE EFFECT OF GENOTYPE OF FEMALES IN THE REALIZATION OF DOMINANT LETHAL MUTATIONS INDUCED IN SPERMATIDS OF MALES. (Rus.) Malashenko, A. M. (Res. Lab. of Experimental Biological Model, Acad. of Medical Sciences of the U.S.S.R., Moscow Region, U.S.S.R.); Surkova, N. I. *Genetika* 11(2):105-111; 1975.

1362 GENETIC ACTIVITY OF 1,2-DIBROMO-3-CHLOROPROPANE, A WIDELY-USED FUMIGANT. (Eng.) Rosenkranz, H. S. (Coll. of Physicians and Surgeons, Columbia Univ., New York, N.Y.); *Bull. Environ. Contam. Toxicol.* 14(1):8-12; 1975.

1363 METABOLISM OF VINYL CHLORIDE. (Eng.) Bolt, H. M. (Univ. Inst. Toxicology, D-74 Tübingen, Wilhelmstrasse 56, West Germany); Kappus, H.; Buchter, A.; Bolt, W. *Lancet* 1(7922): 1425; 1975.

1364 A SIMPLE, SENSITIVE DETERMINATION AND IDENTIFICATION OF VINYL CHLORIDE BY GAS CHROMATOGRAPHY WITH A HALL DETECTOR. (Eng.) Ernst, G. F. (Food Inspection Service, Nyenoord 6, Utrecht, The Netherlands); Van Lierop, J. B. H. *J. Chromatogr.* 109(2):439-440; 1975.

See also:

* (Rev): 1201, 1202, 1203, 1204, 1205, 1206, 1207, 1208, 1223, 1224, 1225, 1226, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1252, 1253
* (Phys): 1373
* (Viral): 1413, 1420
* (Immun): 1500, 1501, 1526, 1527, 1541, 1556, 1557, 1560, 1573
* (Path): 1617, 1619, 1623, 1630, 1631, 1655, 1693
* (Epid-Biom): 1711, 1713, 1714, 1715, 1718, 1721, 1722, 1723, 1725, 1732

5 THOROTRAST AND THE LIVER: A REMINDER. (Eng.) Selinger, M. (Veterans Admin. Hosp., South Huntingdon Ave., Boston, Mass. 02130); f, R. S. *Gastroenterology* 68(4):799-803; 1975.

case of a 61-yr-old man who had received Thoro-st for arteriography 23 yr previously and who sequently developed the di Guglielmo syndrome reported. The patient suffered from progressive kness, fatigue and pancytopenia. The liver was arged and firm and the spleen was not palpated. iver-spleen scan failed to visualize the spleen no defects were noted in the liver. A periphe-blood smear revealed spur, helmut and target ls, and approximately 200 nucleated RBC/100 WBC. e marrow examination was interpreted as consis-t with the clinical impression of the di Guglielmo drome. Liver function tests were within normal its except for minimal elevation of serum gluta--oxalacetic transaminase (52-59 U). Despite treat-t with androgens and transfusions of blood and telets, the patient had intermittent epistaxis gastrointestinal bleeding. Massive upper gastro-estinal hemorrhage was the terminal event. Post-tem examination revealed the presence of a large ntity of blood in the intestinal lumen. Multiple phageal and gastric erosions were the probable rce of bleeding. Aggregates of Thorotrast were nd in the fibrotic liver, spleen, neck, esophagus, icardium and in a fibrotic pulmonary nodule. Ten ths later, a second patient was admitted with a phoma associated with Thorotrast administration yr earlier. The properties, uptake, distribution, localization of Thorotrast are reviewed, and atic neoplasia caused by Thorotrast is discussed.

6 EFFECT OF IODODEOXYURIDINE ON TUMOR INDUC-TION IN X-IRRADIATED RATS. (Eng.) Myers, K. (Chalk River Nuclear Laboratories, Atomic En-y of Canada Ltd., Chalk River, Ontario, Canada). *erientia* 31(6):687-688; 1975.

determine whether or not 5-iododeoxyuridine (IUdR) reases the carcinogenic effects of x-irradiation rats, male and female blackhooded Collip rats were ided into groups and treated with one of the fol-ing: (a) five ip injections of 500 mg/kg IUdR ov-a 5-wk period (b) five whole-body exposures of 165 rradiation (300 kVep), or (c) five injections of R plus five exposures of 165 R. IUdR treatment not appear to interfere with the growth of the mals or to induce tumor development. However, R markedly increased the short-term lethal effects x-irradiation; 34 of 65 animals died within 60 s of exposure to both agents compared with 1 of 66 eated with x-ray alone. IUdR also resulted in ked interference with the growth of animals sur-ing the combined treatment. However, the agent not increase the incidence of mammary tumors, kemia plus lymphomas, or miscellaneous tumors in-ced by x-irradiation. These results differ from se obtained with agents such as urethane or fluo-ylenebisacetamide, which alter the pattern of tu-r development in animals treated with ionizing ir-iation. The difference may reflect the fact that dR is selectively incorporated into the DNA of only those cells that are actively synthesizing DNA at the time of injection.

1367 CENTROMERIC ASYMMETRY AND INDUCTION OF TRANSLOCATIONS AND SISTER CHROMATID EX-CHANGES IN MOUSE CHROMOSOMES. (Eng.) Lin, M. S. (Children's Hosp. Medical Center, Boston, Mass. 02115); Davidson, R. L. *Nature* 254(5498):354-356; 1975.

Radiation- and drug-induced chromosome translocation and sister chromatid exchange were investigated in mouse cells from two permanent lines, RAG and A9. The relationship between DNA polarity and chromosomal rejoining in translocations induced by x-rays was examined using the fluorometrically detectable centromeric asymmetry as a cytological marker for the chain of DNA with a given polarity. End-to-end translocations were not seen in nonirradiated RAG cells; but one generation after irradiation (350 rads), end-to-end translocations appeared in more than 25% of metaphases. Both telo-dicentric and telo-meta-dicentric chromosomes were observed. Analysis of nonirradiated A9 cells revealed a stable chromosome aberration in more than 90% of the cells. After one cycle of replication in 5-bromodeoxyuridine (BrdU, 10^{-5}M), the findings indicated that DNA polarity was involved in determing the orientation of chromosomes during end-to-end and end-to-centromere translocations. In RAG cells grown for one generation in BrdU, sister chromatid exchange within the centromeric region occurred with a very low frequency (0.05/metaphase); after one cycle of replication in mitomycin C (MC, 0.05 μg/ml) plus BrdU, the frequency increased to 11.2/metaphase. In contrast to the centromeric region, sister chromatid exchange in the noncentromeric region was not observed until the second cycle after replication in MC plus BrdU. This suggested that sister chromatid exchange involves exchanges of double-stranded DNA molecules rather than exchanges of single polynucleotide strands. The ability to detect sister chromatid exchange in the centromeric region at the first metaphase when MC and BrdU are added together is attributed to the asymmetric distribution of thymidine in the two strands of mouse satellite DNA. This natural asymmetry thus provides a system in which sister chromatid exchange can be detected within the first generation after labeling, which could facilitate certain types of studies on chromosome breakage and rejoining.

1368 EXTERNAL DOSE ESTIMATES FOR FUTURE INHABI-TANTS OF ENIWETOK ATOLL. (Eng.) Gudiksen, P. H. (Lawrence Livermore Lab., Univ. California, Livermore, Calif.); Jones, D. E.; Beck, H. L.; Mc-Laughlin, J. E.; Stuart, T. P.; Lynch, O. D. T., Jr. *Nature* 257(5524):284-287; 1975.

Environmental radiation levels were measured on Eniwetok Atoll, a former U.S. nuclear weapons test site, to determine whether or not the former Marshallese inhabitants can safely return. These radiation measurements were studied along with data on distributions and expected future life styles of the population. Unmodified and modified integral 5⁻, 10⁻, 30⁻, and 70-yr γ-ray doses were calculated. The unmodi-

fied calculations reflect corrections made for radioactive decay but not corrections for possible weathering and subsequent deeper penetration of radionuclides in the soil; modified doses take into account the effect of three attempts to reduce the exposure rates on the atoll: (a) covering village areas with uncontaminated coral gravel (b) ploughing the topsoil on Janet, the largest of the more heavily contaminated northern islands; or (c) ploughing the topsoil of all the northern islands. The calculations show that the unmodified 70-yr integral doses are comparable to U.S. values. At most, implementation of the first two modifications should be enough to assure mean population exposures well below the U.S. levels. It is noted, however, that the external dose levels calculated for Janet island are still appreciably higher than corresponding levels found elsewhere in the Marshall Islands. Restricting the permanent villages to 'clean' southern islands at least temporarily would result in lower exposure.

1369 DOSE DISTRIBUTION AND NEOPLASIA IN THE LUNG FOLLOWING INTRATRACHEAL INSTILLATION OF $^{239}PuO_2$ AND ASBESTOS. (Eng.) Sanders, C. L., Jr. (Biol. Dept., Batelle, Pacific Northwest Lab., Richland, Wash. 99352). *Health Phys.* 28(4):383-386; 1975.

The influence of dose distribution on pulmonary carcinogenesis was examined in four groups of female Wistar SPF rats given an intratracheal instillation of either saline, 0.9 mg asbestos, 30 nCi $^{239}PuO_2$, or mixed 0.9 mg asbestos + 50 nCi $^{239}PuO_2$ particles. The pulmonary retention half-time for ^{239}Pu was about 200 days in rats given PuO_2 and 450 days in rats given PuO_2 + asbestos; the cumulative radiation dose to lung at two yr after instillation was 400 and 1200 rads, resp. PuO_2 particles were concentrated with asbestos-induced scars in peribronchiolar regions of the lung as compared with a more homogeneous distribution when PuO_2 was given alone. No differences in mortality were seen between any of the groups. At 2 yr after instillation, the incidences of pulmonary carcinoma were none in rats given saline, 4.5% in rats given asbestos, 32% in rats given PuO_2, and 21% in rats given PuO_2 + asbestos. It is concluded that immobilization and concentration of alpha emitters in the lung reduces their carcinogenic effects.

1370 BIOLOGICAL ALTERATIONS RESULTING FROM CHRONIC LUNG IRRADIATION. II. CONNECTIVE TISSUE ALTERATIONS FOLLOWING INHALATION OF ^{144}Ce FUSED CLAY AEROSOL IN BEAGLE DOGS. (Eng.) Pickrell, J. A. (Lovelace Foundation for Medical Education and Res., Albuquerque, N.M. 87108); Harris, D. V.; Pfleger, R. C.; Benjamin, S. A.; Belasich, J. J.; Jones, R. K.; McClellan, R. O. *Radiat. Res.* 63 (2):299-309; 1975.

Beagle dogs were exposed by inhalation to an aerosol of ^{144}Ce clay to quantitate the relationship between pulmonary radiation dose and induced fibrosis. Collagen, elastin, glucosamine, and the ratios of elastin/collagen, hydroxyproline/hydroxylysine, and hydroxyproline/proline were determined to indicate changes in connective tissue constituents. Total lung collagen was partitioned into native collagen, soluble collagen, and ultrafilterable hydroxyproline peptides. Increased total lung collagen correlated best with increasing cumulative radiation dose and increasing time after inhalation exposure. The increase in total lung collagen was not seen until more than four months after exposure and a cumulative dose of about 40,000 rad. Soluble collagen and low-molecular weight hydroxyproline peptide quantities both increased two months after exposure and cumulative doses of 20,000-27,000 rad. A variable elastin response apparently was not related to either increasing time or increasing radiation dose after exposure. These results indicate that collagen accumulation is an important factor in pulmonary fibrosis. Although collagen synthesis and breakdown were both activated at a relatively early time after inhalation, a significant increase in native collagen (scarring) occurred only when the metabolic balance was altered by protracted time or irradiation after exposure. The interrelationships observed in this study provide insight into the mechanism of fibrosis induced by chronic pulmonary injury.

1371 A METHOD FOR INVESTIGATING THE METABOLISM OF THE TRANSPORTABLE FRACTION OF PLUTONIUM AEROSOLS. (Eng.) Stather, J. W. (Natl. Radiol. Prot. Board, Res. and Development Div., Harwell, Didcot, Oxon, England); Howden, S.; Carter, R. F. *Phys. Med. Biol.* 20(1):106-124; 1975.

The metabolism of the transportable fraction of soluble and insoluble forms of plutonium following their deposition in the respiratory system of 7-8-wk-old inbred rats by either inhalation or pulmonary intubation was investigated. The inhalation experiments were performed with aerosols of either plutonium dioxide or mixtures of plutonium and sodium oxides in which 90% of the aerosol mass was in particles having aerodynamic diameters of 0.5-2.5 µm. Exposure lasted for 4-5 min. In another series of experiments, measured aliquots of solutions or suspensions of plutonium were administered to animals by intubation into the pulmonary region of the lungs. The plutonium was intubated in several chemical forms: plutonium dioxide, plutonium nitrate, plutonium citrate, and a mixture of plutonium-sodium oxides in various ratios. The rats were sacrificed at intervals ranging from ten minutes to three months after exposure and the lung clearance and tissue retenion of plutonium was measured. Mixed aerosols of plutonium dioxide and sodium oxide were more transportable in the lung than aerosols of plutonium dioxide alone. A maximum transportability was reached at a Pu:Na atomic ratio of about 1:20, when the transportable fraction of plutonium was 45 times that from a plutonium dioxide aerosol alone. The transportable fraction varied considerably with the chemical form of the plutonium. One week after pulmonary intubation of a solution of plutonium citrate, the extrapulmonary tissue deposit was 69% of the initial pulmonary deposit; in the case of a suspension of plutonium dioxide, the corresponding value was only 0.075%. The metabolism of plutonium following its entry into the systemic circulation, however, was largely independent of the original chemical form deposited in the lung. The liver accumu-

ated only about 16% of the activity deposited in issues from the blood, implying that plutonium was irculating in the blood predominantly in a monoeric form. The cumulative excretion of plutonium n the urine over the first week after pulmonary eposition as either the dioxide, citrate, or nirate was equivalent to about 4.5% of the extraulmonary tissue deposit; the results suggest that his value could be used as a basis for calculating he activity deposited in tissues from the blood in an. The finding that approximately one-third of he activity passing through membranes with a pore ize of 100 nm or less was deposited in extrapulonary tissues at one week after sodium citrate inubation suggests that a fair assessment of the soluility of a plutonium aerosol can be obtained by iltration techniques alone.

1372 DIGESTIVE TRACT CANCERS AND THOROTRASTOSIS. (Fre.) Hepp, J. (Hopital Americain, 92200 Neuilly-sur-Seine, Paris, France); Bismuth, H.; Franco, D. *Lyon Chirurg.* 71(2):81-84; 1975.

1373 CO-CARCINOGENESIS IN THE RAT GASTROINTESTINAL SYSTEM [abstract]. (Eng.) Vogel, H. H., Jr. (Univ. Tennessee Cent. Health Sci., Memphis); Sebes, J. I. *Radiat. Res.* 62(3):596-597; 1975.

1374 BACKGROUND RADIATION AS A CARCINOGENIC HAZARD [abstract]. (Eng.) Frigerio, N. A. (Argonne Natl. Lab., Ill.); Eckerman, K. F.; Stowe, R. S. *Radiat. Res.* 62(3):599; 1975.

1375 RADIOBIOLOGICAL STUDIES OF A MURINE LEUKEMIA [abstract]. (Eng.) Evans, T. C. (Radiat. Res. Lab., Univ. Iowa, Iowa City); Eklund, S.; Crouse, D.; Brandt, K.; Ainsworth, J. *Radiat. Res.* 62(3):550; 1975.

1376 EVIDENCE OF ONCOGENIC COFACTORS IN RADIATION-INDUCED TUMORS IN MICE. (Eng.) Patricio, M. B. (Centro de Estudo de Medicina Nuclear do Instituto de Alta Cultura, Laboratorio de Isotopos e Servico de Radioterapia do Instituto Portugues de Oncologia de Francisco Gentil, Lisboa, Portugal); Clode, W. H.; Ricardo, J. A. *J. Surg. Oncol.* 7(1): 57-61; 1975.

1377 POST-IRRADIATION MEMBRANE ALTERATIONS OF L5178Y AND B16 MELANOMA CELL LINES [abstract]. (Eng.) Gersten, D. M. (Univ. Rochester, N.Y.); Bosmann, H. B. *Radiat. Res.* 62(3):584-585; 1975.

1378 DELAYED X-RAY-INDUCED DEPRESSION OF DNA SYNTHETIC RATE IN HeLa CELLS [abstract]. (Eng.) Saha, B. K. (Washington Univ. Sch. Med., Saint Louis, Mo.); Tolmach, L. J. *Radiat. Res.* 62(3):586; 1975.

1379 CELL KINETICS OF IRRADIATED EXPERIMENTAL TUMORS: CELL TRANSITION FROM THE NONPROLIFERATING TO THE PROLIFERATING POOL DURING REGENERATION [abstract]. (Eng.) Goldfeder, A. (Cancer Radiobiol. Res. Lab., New York Univ., N.Y.); Potmesil, M. *Radiat. Res.* 62(3):527; 1975.

1380 CELL KINETICS OF IRRADIATED EXPERIMENTAL TUMORS: RELATIONSHIP BETWEEN THE PROLIFERATING AND THE NONPROLIFERATING POOL [abstract]. (Eng.) Potmesil, M. (Cancer Radiobiol. Res. Lab., New York Univ., N.Y.); Ludwig, D.; Goldfeder, A. *Radiat. Res.* 62(3):527-528; 1975.

1381 GROWTH RATE, CELL CYCLE KINETICS, AND TRANSPLANTABILITY OF C3HBA MAMMARY TUMORS REGROWING AFTER X-IRRADIATION [abstract]. (Eng.) Nelson, J. S. R. (Univ. Washington Hosp., Seattle). *Radiat. Res.* 62(3):528; 1975.

See also:

* (Rev): 1226, 1252
* (Viral): 1455, 1468
* (Immun): 1548, 1560, 1563
* (Path): 1618, 1623, 1640, 1645, 1678
* (Epid-Biom): 1716, 1724

1382 INTERMEDIATE IN ADENOVIRUS TYPE 2 REPLICATION. (Eng.) Pearson, G. D. (Dept. Biochemistry and Biophysics, Oregon State Univ., Corvallis, Oregon 97331). *J. Virol.* 16(1):17-26; 1975.

The complex containing replicating adenovirus chromosomes was studied, and an analysis of nascent chains in replicating molecules is presented. Using HeLa S_3 cells grown in suspension culture, inocula for all experiments consisted of type 2 adenovirus purified in CsCl density gradients. HeLa cells were synchronized by two successive exposures to 2 mM thymidine and infected with type 2 adenovirus at the beginning of the S phase; infected cells did not enter mitosis or initiate a second round of cellular DNA synthesis. Intermediate DNA was demonstrated in the replication complex; a CsCl-ethidium bromide density gradient revealed that intermediate DNA had an increased buoyant density (1.725) as compared to mature DNA (1.715), and a greater sedimentation coefficient of 100S *vs* 31S. Digestion of intermediate DNA with S_1 endonuclease, but not with RNase, abolished the difference in densities. Kinetic studies and alkaline velocity sedimentation revealed that no single strands longer than unit (34S) were ever detected; the time to complete unit length single strands was calculated as 16 min. The complex was insensitive to detergents, yet experience an altered sedimentation rate due to changes in ionic strength. The intermediate DNA sedimented at 10S, corresponding to a chain 1,750 nucleotides long; other nascent strands representing integral multiples were also found. Although nascent (i.e. intermediate) DNA contained in the adenovirus replication complex was shown to differ physically from mature viral molecules, the newly finished molecules still contained single-strand interruptions, requiring an additional 15-20 min for complete joining of daughter strands. The author speculates that unjoined strands may be sealed at the junctions between origins and termini.

1383 TUMOUR ANTIGEN SPECIFICITY OF A DNA-BINDING PROTEIN FROM CELLS INFECTED WITH ADENOVIRUS 2. (Eng.) Gilead, Z. (St. Louis Univ. Sch. Med., Mo.); Arens, M. Q.; Bhaduri, S.; Shanmugam, G.; Green, M. *Nature* 254(5500):533-536; 1975.

A new DNA-binding polypeptide of molecular weight 75,000 (75k protein) in cells infected with adenovirus 2 (Ad 2) was studied by three immunological methods. Most of the 75k protein purified from infected cell cytoplasm by single-stranded DNA-cellulose column chromatography was present in the fraction eluting in 0.6 M NaCl. Unlabeled 0.6 M eluates were assayed by complement fixation using hamster sera against several tumor antigens. Positive fixation to a titre of 1:32 occurred with anti-Ad 2 and anti-Ad 1 tumor sera. Anti-Ad 12 tumor serum, anti-SV40 tumor serum, and goat antiserum to purified Ad 2 virions were all negative. Controls using mock-infected cells were negative. An indirect radioimmune precipitation assay showed a maximum 63-66% of the input radioactivity of the labeled 0.6 M eluate precipitated with anti-Ad 1 and anti-Ad 2 immunoglobulins, whereas only 7%-10% was precipitated by the immunoglobulins from the anti-SV40 or goat anti-Ad 2 virion sera. In radioimmune-inhibition experiments, the precipitation of ^{3}H-labeled protein from the 0.6 M eluate with a saturating level of anti-Ad 2 tumor immunoglobulin could be inhibited by 80% by preincubating the immunoglobulin with increasing amounts of unlabeled 0.6 M eluate or unlabeled infected cytoplasm. The results indicate that the 75k DNA-binding protein is a non-virion, group C-specific tumor antigen found only in infected cells.

1384 ADENOVIRUS TYPE 2 DNA REPLICATION. I. EVIDENCE FOR DISCONTINUOUS DNA SYNTHESIS. (Eng.) Winnacker, E. L. (Inst. Genet., Univ. Cologne, West Germany). *J. Virol.* 15(4):744-758; 1975.

The characteristics of viral DNA synthesis in isolated nuclei from adenovirus 2 (Ad2)-infected HeLa cells are described. Human HeLa cells were infected with Ad2 virus. After 20 hr, the nuclei were isolated. DNA-DNA hybridization and fragmentation by Eco RI restriction endonuclease were observed. Maximum incorporation of [^{3}H]dTTP into acid-insoluble material occurred 16-22 hr after infection in isolated nuclei. Over 90% of incorporated radioactivity banded at densities typical of viral DNA. From DNA-DNA hybridization, it was determined that newly synthesized DNA was virus-specific. Isolated nuclei reflected viral DNA replication as it occurs in infected cells. Fragments of 10S were observed to be intermediates in viral DNA synthesis in the isolated nuclei, indicating discontinuous DNA synthesis along parts of the Ad2 genome. The results suggest that a ribonucleoside-dependent initiation step, as well as two DNA polymerase-catalyzed reactions, are involved in the discontinuous replication of Ad2 DNA.

1385 ADENOVIRUS DEOXYRIBONUCLEIC ACID REPLICATION. II. SYNTHESIS OF VIRAL DEOXYRIBONUCLEIC ACID *IN VITRO* BY A NUCLEAR MEMBRANE FRACTION FROM INFECTED KB CELLS. (Eng.) Yamashita, T. (St. Louis Univ. Sch. Med., Mo.); Arens, M.; Green, M. *J. Biol. Chem.* 250(9):3273-3279; 1975.

The enzymological properties of an isolated nuclear membrane fraction in association with newly synthesized adenovirus 2 DNA were studied. This "DNA replication complex" was isolated on a discontinuous sucrose gradient. The complex isolated from adenovirus 2-infected KB cells possessed seven times the endogenous DNA polymerase activity of the complex from uninfected KB cells. Optimization of activity was at pH 7.5-8.5 in the presence of 50 mM KCl, 2 mM ATP, 10-20 mM Mg^{2+}; all four nucleotide triphosphates were required for activity. RNase A inhibited the reaction, suggesting an involvement of RNA synthesis in DNA replication. The adenovirus 2 DNA complex hybridized 48% with adenovirus 2 DNA immobilized on membrane filter; this value was only 1% with hybridization to KB cell DNA; the DNA sequences were synthesized *in vitro*. On neutral sucrose density gradients, the *in vitro*-synthesized DNA sedimented at 18S. Using equilibrium centrifugation, labeled DNA shifted from

the LL region toward the hybrid density region indicating a semiconservative replication. An RNA-initiated adenovirus 2 DNA synthesis occurred *in vivo*, and elongation occurred *in vitro*. The interpretation of this data is uncertain because of the potential artifacts associated with the experimental methods used.

1386 COVALENT INTEGRATION OF VIRAL DNA INTO CELL DNA IN HAMSTER CELLS TRANSFORMED BY AN AVIAN ADENOVIRUS. (Eng.) Bellett, A. J. D. (John Curtin Sch. Med. Res., Australian Natl. Univ., Canberra). *Virology* 65(2):427-435; 1975.

A method of quantitative analysis for viral DNA integration is described for a kinetic study of integration in nonpermissive, transformable cells. Chick embryo lethal orphan (CELO) virus was grown in chick embryo kidney cells with 1 mCi ^{32}P per culture of 10^7 cells. The virus was purified and its DNA was extracted. It was fragmented to 400 nucleotides (6.6S). DNA was extracted from hamster skin cells transformed by CELO virus (THS cells) and fragmented to less than 500 nucleotides. ^{32}P-labeled CELO virus DNA fragments (20-80 ng) were mixed with DNA fragments from THS cells or normal hamster cells (BHK), denatured, and allowed to reassociate. The fraction of ^{32}P DNA remaining single-stranded was plotted against time. The slopes obtained for reassociation of CELO virus DNA in presence of THS-DNA and BHK-DNA were used to calculate the amount of viral DNA in the sample of THS-DNA. A mean of 15.7 ng of CELO virus DNA per mg of THS cell DNA was obtained and standard error reduced to 3.4 ng. Reassociation of denatured CELO virus DNA in the presence of THS cell DNA showed that transformed cells contain viral DNA. All the viral DNA present in THS cells was integrated into cell DNA by alkali-resistant bonds.

1387 POLYPEPTIDES ISOLATED FROM RIBOSOME-LIKE STRUCTURES OCCLUDED IN AVIAN MYELOBLASTOSIS VIRUS (AMV). *(Eng.)* Michlová, A. (Inst. Organic Chemistry and Biochemistry, Czechoslovak Acad. Sciences, 166 10 Prague, Czechoslovakia); Poláková, K.; Říman, J. *Neoplasma* 22(2):123-132; 1975.

The protein composition and antigenic properties of ribosome-like particles isolated from avian myeloblastosis virus (AMV) were determined by acrylamide gel electrophoresis and by the immunodiffusion technique. Virus was isolated from the heparin-treated plasma of leukemic chickens, and ribosome-like particles and proteins were isolated from the cytoplasma of chick leukemic myeloblasts and normal chick liver cells. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed significant differences between the protein composition of AMV virions and that of virus-occluded ribosomes (VRs). VRs contained characteristic high molecular wt proteins (68,000-140,000), and almost no AMV components. Further differences were observed between the proteins of the VRs and whole AMV in the molecular wt range of 33,000-39,000. The protein spectrum of VRs showed differences in the protein composition of cytoplasmic ribosomes from leukemic chick myeloblasts and from normal chick liver cells. The main protein component had a molecular wt range of 13,000-70,000. Immunological studies employing Ouchterlony's mircotechnique revealed the presence of antigenic determinants characteristic of AMV, suggesting gs antigens. VRs resemble the ribosomal precursors of HeLa cells in their protein spectrum, and they differ strongly from the cytoplasmic ribosomes studied.

1388 A COMPARATIVE STUDY OF THE AVIAN RETICULOENDOTHELIOSIS VIRUS: RELATIONSHIP TO MURINE LEUKEMIA VIRUS AND VIRUSES OF THE AVIAN SARCOMA-LEUKOSIS COMPLEX. (Eng.) Moelling, K. (Robert Koch Institute, Berlin, West Germany); Gelderblom, H.; Pauli, G.; Friis, R.; Bauer, H. *Virology* 65(2):546-557; 1975.

Reticuloendotheliosis virus was characterized and compared with typical avian and murine C-type RNA tumor viruses. Electron microscopy revealed that mature particles of reticuloendotheliosis virus were very similar to murine leukemia virus particles, but quite different from those of avian sarcoma-leukosis virus. The properties of the purified DNA-polymerase of these viruses were analyzed; although the enzyme from reticuloendotheliosis virus did not show the characteristic properties of DNA-polymerase from a virus of avian origin, it did demonstrate a number of similarities to the murine virus enzyme. Apart from such structural and biochemical analogies, no direct relation between reticuloendotheliosis virus and murine leukemia virus was established. Infectivity of reticuloendotheliosis virus in two strains of mouse cells could not be demonstrated. Immunodiffusion tests for reaction of purified, disrupted reticuloendotheliosis virus with antisera specific for avian sarcoma-leukosis virus structural components or for the interspecies-specific reaction characteristic of mammalian RNA tumor virus p30 protein were uniformly negative. The authors conclude that reticuloendotheliosis virus must be considered a distinct group of avian RNA tumor viruses. These viruses have significant structural similarities to mammalian viruses, but nonetheless differ in thei antigenic determinants.

1389 REASSOCIATION OF 4 S AND 5 S RNA'S WITH THE GENOME OF AVIAN SARCOMA VIRUS. (Eng.) Taylor, J. M. (Dep. Microbiol., Univ. California, San Francisco); Cordell-Stewart, B.; Rohde, W.; Goodman, H. M.; Bishop, J. M. *Virology* 65(1):248-259; 1975.

A tryptophan tRNA derived from the host cell serves as primer for the initiation of DNA synthesis by the reverse transcriptase of avian sarcoma virus (ASV). The binding of primer to the viral genome was characterized by studying the reassociation of low molecular weight RNA's with high molecular weight subunits of ASV-RNA. Primer can be annealed to two to four sites on the viral genome to reconstitute a template fully active with reverse transcriptase. The annealing is specific and accurate; none of the other 4 S RNA's found in ASV will reassociate with viral genome under any of the conditions tested,

and the reconstructed complex denatures with the same T_m as the native complex between primer and viral genome. These results substantiate our conclusion that the primer in ASV is a single species of tRNA. Chicken ribosomal 5 S RNA also reanneals to the ASV genome, presumably by binding to the sites originally occupied by 5 S RNA in viral 70 S RNA.

1390 R-TYPE VIRUS-LIKE PARTICLES IN AVIAN SARCOMA VIRUS-INDUCED RAT CENTRAL NERVOUS SYSTEM TUMORS. *(Eng.)* Cloyd, M. W. (Duke Univ. Medical Center, Durham, N.C. 27710); Burger, P. C.; Bigner, D. D. *J. Natl. Cancer Inst.* 54(6): 1479-1482; 1975.

Avian sarcoma virus (ASV)-induced rat CNS tumors were studied with the light and electron microscope in order to assess the presence of morphologically detectable virus and to classify the tumor type. Eight-wk-old rats bearing the Bratislava 77, subgroup C ASV-induced tumor were vascularly perfused. The four cerebral tumors studied were astrocytomas; these consisted of large cells of occasional gemistocytic appearance with closely interwoven processes, nexuses, and abundant 80-A intracytoplasmic filaments. The single spinal cord tumor examined was interpreted as a schwannoma. At both light and electron microscopic levels, the CNS tumors appeared generally similar to those previously described. However, the virus-like particles (VLP) differed from most reported R-type VLP; these were usually clustered near, but not within, cisternae of rough endoplasmic reticulum, and within the cell cytoplasmic matrix. No membranous envelopes were observed. VLP in these tumors differ from most reported R-type VLP. The author suggests that the R-type VLP may have originated from an endogenous hamster VLP. Alternatively, the VLP may be widely distributed among several species, including the rat, and may be related to cell transformation.

1391 ALTERATIONS IN MEMBRANE POLYPEPTIDES OF CHICK EMBRYO FIBROBLASTS INDUCED BY TRANSFORMATION WITH AVIAN SARCOMA VIRUSES. (Eng.) Isaka, T. (Fac. Pharm. Sci., Univ. Tokyo, Japan); Yoshida, M.; Owada, M.; Toyoshima, K. *Virology* 65(1):226-237; 1975.

Membrane proteins of chick embryo fibroblasts (CEF) transformed with various strains of avian sarcoma viruses were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels and compared with those of untransformed cells. Four changes were consistently detected in CEF transformed with B77, the Prague strain of Rous sarcoma virus (PR-RSV) or the Schmidt-Ruppin strain of RSV (SR-RSV). These included the appearance of a polypeptide band of about 90,000 molecular wt, an increase in amount of a 79,000 dalton polypeptide, and significant decreases in a protein and polypeptide of 200,000 and 50,000 daltons, respectively. Temperature-sensitive (ts) mutants of these strains, LA334 (of B77), LA31 (of PR-RSV) or OS122 (of SR-RSV), showed similar changes at 36 C. At 41 C, the profiles of the membrane proteins were similar to those of uninfected cells; this was true except in the case of the decrease of the 200,000 dalton protein. The appearance of the 90,000 molecular wt polypeptide and the decrease in the 50,000 dalton polypeptide were reversible and clearly observable within a few hours after a temperature shift of CEF infected with ts mutants. Fusiform transformation induced by a variant of B77 was also shown to induce these two alterations. From these and other results, the appearance of the polypeptide band of 90,000 daltons, which could not be detected in untransformed cells, and the marked decrease in amount of a protein of 50,000 daltons in cell membranes were concluded to be closely correlated with transformation of CEF.

1392 MAPPING RNase T_1-RESISTANT OLIGONUCLEOTIDES OF AVIAN TUMOR VIRUS RNAs: SARCOMA-SPECIFIC OLIGONUCLEOTIDES ARE NEAR THE POLY(A) END AND OLIGONUCLEOTIDES COMMON TO SARCOMA AND TRANSFORMATION-DEFECTIVE VIRUSES ARE AT THE POLY(A) END. (Eng.) Wang, L.-H. (Dept. Molecular Biology, Univ. California, Berkeley, Calif. 94720); Duesberg, P.; Beemon, K.; Vogt, P. K. *J. Virol.* 16(4):1051-1070; 1975.

The large RNase T_1-resistant oligonucleotides of the nondefective (nd) Rous sarcoma viruses (RSV), Prague RSV of subgroup B (PR-B), PR-C, and B77 of subgroup C; their transformation-defective (td) deletion mutants, td PR-B, td PR-C, and td B77; and replication defective (rd) RSV(-) were completely or partially mapped on the 30-40S viral RNAs. The location of a given oligonucleotide relative to the poly(A) terminus of the viral RNAs was directly deduced from the smallest size of the poly(A)-tagged RNA fragment from which it could be isolated. Identification of distinct oligonucleotides was based on their location in the electrophoretic/chromatographic finger-print pattern and on analysis of the RNase A-resistant fragments. The number of large oligonucleotides per poly(A)-tagged fragment increased with increasing fragment size. Three sarcoma-specific oligonucleotides were identified in the RNAs of PR-B, PR-C, and B77 by comparison with the RNAs of the corresponding td viruses. The sarcoma-specific oligonucleotides of these three sarcoma viruses had very similar compositions. Based on the map positions of the oligonucleotides, the sarcoma-specific sequences of the nd viral RNAs were estimated to map 6.6-20% away from the poly(A) end. The oligonucleotide maps of the RNAs of nd and td PR-B were the same, with the exception of the sarcoma-specific sequences, as were the maps of the nd and td B77 RNAs. Comparisons of the map locations of the oligonucleotides from the three nd sarcoma virus strains suggested that homologous oligonucleotides are found in certain homologous map positions of the three respective RNAs. The complexity of the PR-C RNA was 3.22×10^6 daltons and that of B77 was 3.02×10^6daltons. The RNase T_1-resistant oligonucleotides of poly(A)-tagged RNA fragments ranging up to 15S from each nd/td virus pair were similar. The poly(A)-tagged 12S RNA fragments of all avian tumor viruses shared one oligonucleotide, "spot C", which mapped very near the poly(A) end. The poly(A) terminal sequences of the

/td virus RNAs started with poly(A), continued th a heteropolymeric sequence shared partially or mpletely by nd and td viruses, and further connued with sarcoma-specific sequences in the case the nd sarcoma virus RNAs. Further experimentaon is needed to determine the mechanism of generion of deletion mutants from nd viruses.

93 CLONAL VARIATION OF THE MOUSE CELLS IN THE ENDOGENOUS C-TYPE VIRUS INDUCTION BY 5-DO-2'-DEOXYURIDINE. (Eng.) Yoshikura, H. (Instit du Radium Biologie, Faculté des Sciences, Bâtient 110, 91405 Orsay, France). *J. Gen. Virol.* 28):179-184; 1975.

ta are presented to show that clonal variation ists in the case of C-type virus induction by 5-do-2'-deoxyruidine (IUdR). Using an MLg cell line iginating from a single newborn ddY mouse, N-troic C-type virus able to infect the cells of origin s induced by IUdR. All the clones isolated from e MLg cell line released the virus after IUdR-reatment. There was more than a 100-fold differnce in the virus inducibility between the highly d lowly inducible clones. The [^{125}I]-IUdR inrporation was, however, the same for both types f clones. When the induced virus was titrated in e S + L- C182 cells, the XC plaque titer was about en-fold higher than murine sarcoma virus rescue ocus titer. It is suggested that, in the case of ouse tropic virus at least, the expression of the ndogenous virus genomes is not suppressed by a arge amount of repressor substance, or, if the epressor exists, that some mechanism also exists hich prevents the repressor from acting on the rovirus of the induced virus genomes.

394 ISOLATION OF TYPE C VIRIONS FROM A NORMAL HUMAN FIBROBLAST STRAIN. (Eng.) Panem, . (Dept. Pathology, Univ. Chicago, Chicago, Ill. 0637); Prochownik, E. V.; Reale, F. R.; Kirsten, W. . *Science* 189(4199):297-299; 1975.

ype C virions were spontaneously released from a iploid human fibroblast cell strain after the cells ere serially cultured for six months. Despite heir conversion to virion releasers, the cells aintained a modal distribution of 46 normal chromoomes throughout their life span in culture. Thinection electron microscopy of the cells revealed ypical type C virions, both free in the extracelular space and budding from plasma membranes. The irions incorporated ^{3}H-uridine, banded at a density f 1.17-1.18 g/ml in sucrose equilibrium gradients, nd contained reverse transcriptase which utilizes oth polyadenylate·oligodeoxythymidylate and virion NA as templates. In a double diffusion test, a omponent of the virions reacted strongly with antierum to the interspecies-specific antigen (p30) of a simian sarcoma virus (SiSV), indicating that the irions are antigenically related to SiSV. Comparable amounts of antiserum to the Rauscher mouse leukemia virus p30 and the interspecies-specific antigen (p27) of Rickard feline leukemia virus did not produce precipitin lines with the virions. Antiserum to reverse transcriptase of gibbon ape leukemia virus inhibited the reverse transcriptase of the putative human virions and that of SiSV, but had no effect on the corresponding enzymes of avian or murine RNA tumor viruses. This is apparently the first documented isolation of type C virions from a diploid human cell strain.

1395 KINETICS OF MURINE TYPE C VIRUS-SPECIFIC DNA SYNTHESIS IN NEWLY INFECTED CELLS. (Eng.) Lovinger, G. G. (Flow Laboratories, Inc., Rockville, Md. 20852); Klein, R.; Ling, H. P.; Gilden, R. V.; Hatanaka, M. *J. Virol.* 16(4):824-831; 1975.

The kinetics of murine type C virus-specific DNA synthesis was studied in mouse embryo fibroblasts (MEF) infected with the Rauscher leukemia virus (RLV) or the RLV pseudotype of Moloney sarcoma virus [M-MSV(RLV)]. The critical time for provirus formation was determined using cytosine arabinoside (araC, 10 μg) added at various intervals after infection. In addition, the kinetics of synthesis of (-) strand DNA was determined by the hybridization of excess purified RLV RNA with tritiated DNA obtained from infected cells, and the kinetics of synthesis of (+) strand DNA was monitored by hybridization of the tritiated single-stranded DNA copy of viral RNA with excess unlabeled cellular DNA. The size of both strands was also determined by velocity centrifugation in alkaline glycerol gradients. The replicating transforming functions of RLV and M-MSV(RLV) in MEF were most sensitive to inhibition by ara-C 30-90 min after infection. The initiation of intracellular RLV DNA synthesis was detected by nucleic acid hybridization within this time interval. Treatment of the infected cells with ara-C abolished RLV DNA synthesis. Peak synthesis of the DNA complementary to the infecting RLV genome, the (-) strand, occurred 40-60 min after infection. During this period, two species of DNA were observed with estimated molecular weights of 0.5×10^5 to 1.0×10^5 and 3×10^6. Peak synthesis of the (+) strand viral DNA occurred 50-70 min after infection. The initial species detected had a molecular weight of 1.5×10^5 to 4.0×10^5, which shifted as a function of time to 3×10^6. Both (+) strand species were initially detected in the cytoplasm, followed by a rapid (10-min interval) appearance of the faster-sedimenting species in the nucleus. The virus-specific (-) and (+) strand DNA species are presumably unintegrated intermediates in provirus formation.

1396 LIPOPOLYSACCHARIDE INDUCES C-TYPE VIRUS IN SHORT TERM CULTURES OF BALB/c SPLEEN CELLS. (Eng.) Moroni, C. (Friedrich Miescher-Institut, Basel, Switzerland); Schumann, G. *Nature* 254(5495):60-61; 1975.

The effect of lipopolysaccharide, a B-cell mitogen, was observed in short term cultures of BALB/c spleen cells. During the first 24 hr, spleen cell cultures from 6-8 week-old male BALB/c mice contained lipopolysaccharide W, *Escherichia coli* 0111:B4 (LPS, 16 μg ml^{-1}), concanavalin A (con A, 4 μg ml^{-1}), phytohemagglutinin-P (PHA, 10 μg ml^{-1}), or no mitogen. The

four groups (LPS, con A, PHA and control) were assayed for reverse transcriptase activity with poly(A)$_n$ x poly(dT)$_{12-18}$ as template primer. Enzyme activity was found in LPS-treated culture supernatants, whereas in control cultures or in con A- and PHA-stimulated cultures no activity was detected. Enzyme induction was reproduced using a highly purified LPS from *Salmonella abortus equi*. The presence of C-type particles in LPS-stimulated cultures was verified using electron microscopy. Typical budding and extracellular virus were observed. It is surprising that an ubiquitous agent such as LPS induces C-type virus; this should be investigated further.

1397 ONCORNAVIRUS-LIKE PARTICLES RELEASED FROM ARGININE-DEPRIVED HUMAN LYMPHOBLASTOID CELL LINES. (Eng.) Kotler, M. (Hebrew Univ.-Hadassah Medical Sch., Jerusalem, Israel); Balabanova, H.; Weinberg, E.; Friedmann, A.; Becker, Y. *Proc. Natl. Acad. Sci. USA* 72(11):4592-4596; 1975.

Type-C RNA tumor virus particles were released from three different human lymphoblastoid cell lines (P_3HR-1, Raji, and 1301) after incubation in arginine-deficient medium. The released virus-like particles were characterized by (a) their ability to band in sucrose gradients at a density of 1.16-1.18 g/ml; (b) the presence of an RNA-directed DNA polymerase activity resembling that of the oncornaviruses; and (c) isolation of cores that band at a density of 1.26-1.27 g/ml in sucrose gradients. Examination of the arginine-deprived human lymphoblastoid cell line strain P_3HR-1 by electron microscopy revealed the presence of C-type particles in the intracellular spaces. The possibility that there was a contamination with other oncornaviruses is unlikely, especially because the virus particles were found in the culture medium only when the cells were deprived of arginine, and not in undeprived cells.

1398 THE DETECTION OF THE EPSTEIN-BARR VIRUS (EBV) NUCLEAR ANTIGEN (EBNA) BY ANTI-COMPLEMENT IMMUNOFLUORESCENCE. IMMUNOGLOBULIN CLASS OF ANTIBODIES AND ROLE OF COMPLEMENT. (Eng.) Liabeuf, A. (Laboratoire d'Immunologie des Tumeurs, Groupe de Recherches INSERM U 136, Centre Hayem, Hopital Saint Louis, 75.010 - Paris, France); Nelson, R. A., Jr.; Kourilsky, F. M. *Int. J. Cancer* 15(4): 533-546; 1975.

The Epstein-Barr virus (EBV)-associated nuclear antigen (EBNA) was detected by anti-complement immunofluoresnce (ACIF) in Raji lymphoblastic cell lines. The mechanism of reaction was explored using immunoglobulin (Ig) fractions of anti-EBNA sera and purified components of complement. Anti-EBNA antibodies were always associated with IgG, but were also detectable in the IgM fraction of four sera. β_{1a}/β_{1c} was detected following previous action of C1, C4, C2, and C3. The classical sequence of C' activation was involved in the ACIF reactions. Anti-EBNA antibody reaction in Raji cell nuclei could also be detected by anti-Ig immunofluorescence with a low level of sensitivity. The same pattern of fluorescence was observed with C4, IgG, C3(β_{1a}/β_{1c}) and anti-EBNA antibodies with the corresponding fluorescein-conjugated reagents. Blocking experiments with FAB'2 anti-EBNA fragments represent the appropriate controls in ACIF reactions for the detection of EBNA. The ACIF method proved to be particularly useful for the detection of human tumor-related antigens, but it is prone to false or negative reactions if not strictly controlled.

1399 PRODUCTION OF LYMPHOID TUMORS IN HAMSTERS BY DIRECT IMPLANTATION OF NORMAL HUMAN LEUKOCYTES. (Eng.) Miyoshi, I. (Okayama Univ. Med. Sch., Japan); Kubonishi, I.; Hayashi, T.; Abe, S.; Uchida, H.; Tsubota, T.; Tanaka, T. *Nature* 254 (5497):272; 1975.

Newborn Syrian hamsters were injected ip with 0.5 to 1 x 10^7 viable leukocytes from peripheral blood of an Epstein-Barr virus (EBV) antibody-positive normal male or from umbilical cord blood with and without EBV infection. Leukocyte implantation was followed by twice weekly ip inoculation of 0.1 ml rabbit antilymphocyte serum against hamster thymocytes. The leukocytes from cord blood were incubated with 1 ml of a filtered freeze-thaw extract of the (EBV+) B95 cell line[2] at 37 C for 2 hr before implantation. All three hamsters transplanted with peripheral leukocytes and two of three hamsters transplanted with cord leukocytes infected with EBV were found to have lymphoid tumors involving the lymph nodes, liver, lungs, and kidneys when killed on days 11-21. No tumors were observed in hamsters transplanted with cord leukocytes not infected with EBV. Enlarged inguinal lymph nodes resulting from transplantation of peripheral leukocytes or EBV-infected cord leukocytes were cultured in medium RPMI 1640 supplemented with 20% fetal calf serum, and two lymphoblastoid cell lines were established from a hamster of each group. The cells began to grow vigorously in about 3-4 wk and have been maintained for 6 mo. These cell lines have a normal diploid human chromosome constitution and are positive for EBV nuclear antigen. Electron microscopy revealed EBV particles in lymphoid cells. These results seem to be analagous with the establishment of lymphoblastoid cell lines *in vitro* from normal human peripheral and cord leukocytes, for which an essential role of EBV has been demonstrated. Successful heterotransplantation of normal human leukocytes into hamsters may be based on the same mechanism as in the *in vitro* system. It is possible that normal human lymphocytes can be transformed *in vivo* by EBV and grow progressively in the immunosuppressed heterologous hosts.

1400 FROG VIRUS 3 REPLICATION: ELECTRON MICROSCOPE OBSERVATIONS ON THE TERMINAL STAGES OF INFECTION IN CHRONICALLY INFECTED CELL CULTURES. (Eng.) Kelly, D. C. (Natural Environment Res. Council, Unit Invertebrate Virology, Univ. Oxford, South Parks Road, Oxford, OX1 3RB, U.K.); Atkinson, M. A. *J. Gen. Virol.* 28(3):391-407; 1975.

Baby hamster kidney (BHK), chick embryo fibroblast (CEF), and fat-head minnow (FHM) cells chronically infected with frog virus 3 (10 plaque-forming U/

ell) were examined by scanning and transmission thin section, freeze fracture and surface replica) lectron microscopy. With minor differences the attern of virus development is similar in all three ell lines. Virus particles were detected in cell uclei which subsequently became degenerate very ate in infection. Three inclusions were associa- ed with frog virus 3 cytoplasmic foci of infection; amella structures, extensive microtubule formation in BHK and FHM cells), and linear crystalline struc- ures. The last two structures may play a role in reating or maintaining the cell rounding cytopathic ffect revealed by scanning electron microscopy. ery late in infection most BHK and FHM, but not CEF, ells are stripped of the plasma membrane. Replicas f frozen fractured BHK cells featured cytoplasmic oci of infection, budding at the plasma membrane, nd showed that at early times when virus is detect- d in the nucleus, the nuclear membranes are intact nd morphologically unaltered. Budding at the plas- a membrane was better resolved by scanning and as urface replicas. This demonstrated that sparse to rofuse localized budding occurred. Frequently virus articles were located singly, or as multiples, at he end of, or along, cytoplasmic protrusions which ccur both on the body of the cells and at the cyto- lasmic/coverslip "interface".

401 REPETITIVE SEQUENCES IN COMPLETE AND DEFECTIVE GENOMES OF *HERPESVIRUS SAIMIRI*. Eng.) Fleckenstein, B. (Institut für Klinische irologie der Universität, Erlangen-Nürnberg, 852 rlangen, West Germany); Bornkamm, G. W.; Ludwig, . *J. Virol.* 15(2):398-406; 1975.

ize and molecular weight, genetic complexity and in- ectivity of different *Herpesvirus saimiri* DNA species ere analyzed in owl monkey kidney monolayer cell ines. DNA was purified by sedimentation in su- rose-sodium dodecyl sulfate gradients and by iso- ycnic centrifugation in CsCl. When purified tri- ium-labeled *H. saimiri* preparations were gently ysed on top of CsCl solutions and spun to equili- rium by preparative runs, only two DNA bands re- resenting H and M DNA were observed. The M gen- me was a double-stranded, linear DNA molecule with a mean contour length corresponding to 89 x 10^6 daltons. The M genome contained about 70% of uni- que sequences (light DNA, 36% guanine plus cyto- sine) and 30% reiterated sequences (heavy DNA, 71% guanine plus cytosine). The H genome was composed of heavy DNA only and was more heterogeneous in size. The sequences in the H genome were up to 40-fold reiterated, indicating defectiveness of this type of genome. The repetitions in the H genome and the M genome cross hybridized almost completely and had identical kinetic complexity (2.8 x 10^6 daltons). DNA infectivity studies by using the calcium phosphate and the DEAE-dextran method gave further evidence that H genomes were defective. No infectious virus was recovered from permissive cells treated with heavy DNA, whereas cells infected with the M genome developed cyto- pathic changes after 11-56 days. Defective H gen- omes were present in the progeny virus two pass- ages after transfection. The results indicate that reiterated sequences are present in complete and defective *H. saimiri* genomes.

1402 *IN VITRO* SYNTHESIS OF DNA IN NUCLEI ISOLATED FROM HERPES SIMPLEX VIRUS-INFECTED CELLS, UNTREATED AND TREATED WITH METABOLIC INHIBITORS. (Eng.) Becker, Y. (Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel); Asher, Y. *Virology* 63 (1):209-220; 1975.

Conditions for *in vitro* nuclear DNA synthesis were determined in nuclei isolated from BSC_1 cells in- fected with herpes simplex virus (HSV) at 10 plaque- forming U/cell. Nuclei isolated from HSV-infected cells are able to synthesize viral and cellular DNA molecules under *in vitro* conditions in the presence of the four deoxynucleoside triphosphates, 6 mM Mg^{2+}, and 8% (w/v) sucrose. Under similar conditions, nuclei from uninfected cells did not synthesize DNA. Analysis of the *in vitro* synthesized DNA molecules released from the nuclei revealed heterogenous DNA species, mostly of low molecular wt, which had the density of viral DNA. Nuclei were isolated from infected cells treated *in vivo* with metabolic inhib- itors, and the *in vitro* DNA synthesis was studied. Nuclei from cells treated with inhibitors which affect DNA (distamycin A and camptothecin) did not synthesize DNA *in vitro*, while nuclei treated with certain other inhibitors (hydroxyurea, cytosine arabinoside or cordycepin) showed a low DNA synthe- sis. Analysis of the DNA products revealed that only cellular DNA was synthesized in nuclei of cells treated with hydroxyurea and cytosine arabinoside.

1403 *IN VITRO* TRANSFORMATION OF HAMSTER CELLS BY HERPES SIMPLEX VIRUS TYPE 2 FROM HUMAN PROSTATIC CANCER CELLS. *(Eng.)* Centifanto, Y. M. (Coll. of Med., Univ. of Florida, Gainesville, Fla.); Zam, Z. S.; Kaufman, H. E.; Drylie, D. M. *Cancer Res.* 35(7):1880-1886; 1975.

The capability of a cell-associated herpes simplex virus type 2 (HSV-2) from a human prostatic carci- noma, to induce *in vitro* transformation of hamster embryo cells was investigated. Primary cultures of Syrian hamster embryo cells, $SV40^2$-transformed WI 38 cells, rabbit kidney cells, and human embry- onic kidney cells were used. The presence of HSV- specific antigens was determined by indirect im- munofluorescence techniques, and the Hellstrom colony inhibition technique was used to determine the ability of the transformed cells to form colo- nies in the presence of HSV-2-sensitized lymphocytes. Inoculations of monolayer cultures of the hamster cells with pieces of the original prostatic cancer tissue led to eventual foci formation, which were developed into the YW-74 cell line. Indirect im- munofluorescence with antisera to type 2 revealed intense perinuclear fluorescence in 95% of the transformed cells; the assignment of the herpes- virus to the type 2 group was further confirmed by reagent specificity studies. Electron microscopic studies of thin sections of the transformed cells demonstrated no viral particles in the cytoplasm or extracellular spaces, yet many virus-like particles

in the perinuclear regions. Colony inhibition tests showed that only HSV-sensitized lymphocytes killed the transformed cells. Karyotypic analysis of transformed hamster cells noted gross numerical alterations, being aneuploid and subtetraploid; all transformed cells contained nine large acrocentric chromosomes and one very small acrocentric (marker) chromosome. Evidence is thus presented for the transformation of hamster embryo cells by tumor-associated HSV-2 from a human prostatic carcinoma.

1404 TEMPERATURE-SENSITIVE HOST RANGE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 2. (Eng.) Koment, R. W. (Milton S. Hershey Med. Cent. Pennsylvania State Univ., Hershey); Rapp, F. *J. Virol.* 15 (4):812-819; 1975.

A system utilizing mutants of herpes simplex virus type 2 (HSV-2) was established to study mechanisms utimately determining the nature of interactions between this virus and host cells. Mutants 69 and 74 were obtained by subjecting parental HSV-2 strain 333 to 90 sec of UV irradiation or to 20 μg 5-bromodeoxyuridine, resp. Mutant 46 was derived after incubating parental virus with 50 μg/ml N-methyl-N-nitro-N-nitrosoguanidine for 60 min and mutant 41 was obtained after similar incubation for 10 min. The four mutants were characterized by failure to replicate and induce cytopathic effects at the nonpermissive temperature (39 C) in hamster embryo fibroblast (HEF) cells. CsCl gradient analysis of 3'-methyl tritiated-thymidine-labeled HEF cultures infected with mutants 74 or 69 showed little or no virus DNA formation in the cells at 39 C. Mutants 46 and 41 induced reduced amounts of DNA in HEF cells at 39 C. The ability of the mutants to depress host cell DNA synthesis to some degree was evident at both restrictive and permissive (33 C) temperatures and was especially marked in mutant 74 which showed 41% depression. Mutant pairs were capable of intracellular complementation in HEF cells at 39 C, supporting the conclusion that a virus function is temperature sensitive. Although the mutants neither replicated nor induced cytopathology in HEF cells at 39 C, they did replicate and induce cytopathology to parental levels in primary rabbit kidney cells under the same conditions. This suggests that a host cell function, absent or nonfunctional in HEF cells, is required for the replication of the mutants and is present in rabbit kidney cells at 39 C. The four mutants thus exhibit both a temperature sensitive virus function and a host regulatory mechanism.

1405 TRANSFORMATION OF MOUSE CELLS AFTER INFECTION WITH ULTRAVIOLET IRRADIATION-INACTIVATED HERPES SIMPLEX VIRUS TYPE 2. (Eng.) Boyd, A. L. (Frederick Cancer Res. Center, Frederick, Md. 21701); Orme, T. W. *Int. J. Cancer* 16(4):526-538; 1975.

A transformed mouse cell line (H238) obtained following the infection of 238 BALB/c mouse cells with UV irradiation-inactivated herpes simplex virus type 2 (HSV-2) is described. The transformed cells produced tumors with a 100% incidence within eight weeks in 6-wk-old syngeneic BALB/c mice inoculated ip with 1 x 10^6 cells. Indirect immunofluorescence (IF) tests revealed the presence of HSV antigens in the transformed cells. Antibodies to HSV-2 were found in the sera of tumor-bearing animals by neutralization and IF techniques. Neither HSV-2 infectious virus nor viral antigens could be detected by the transfer of transformed-cell DNA into permissive cells. The success in accomplishing transformation with HSV-1 and HSV-2, in several laboratories and different virus strains, strengthens the probability that HSV is involved in the transformation event.

1406 ONCOGENESIS BY MAREK'S DISEASE HERPESVIRUS IN CHICKENS LACKING EXPRESSION OF ENDOGENOUS (gs, CHICK HELPER FACTOR, ROUS-ASSOCIATED VIRUS-0) AND EXOGENOUS AVIAN RNA TUMOR VIRUSES. (Eng.) Witter, R. L. (Reg. Poult. Res. Lab., Agric. Res. Serv., East Lansing, Mich.); Lee, L. F.; Okazaki, W.; Purchase, H. G.; Burmester, B. R.; Luginbuhl, R. E. *J. Nat. Cancer Inst.* 55(1):215-218; 1975.

Recently developed chickens free of exogenous avian leukosis virus (ALV) infection, replicating endogenous ALV (Rous-associated virus-0), gs antigen, and chick helper factor were investigated with respect to susceptibility to induction of Marek's disease (MD) by ALV-free MD viruses. Dual infection with Rous-associated virus-2 and MD virus did not significantly alter the character of the MD lesions. Thus, exogenous ALV infection was not a requirement for MD virus-induced oncogenesis. Although participation of endogenous RNA tumor virus genes in MD lesion induction could not be excluded, expression of such genes in MD tumors as gs antigen was not established.

1407 INTERMITTENT SPHERING OF VIRUS-TRANSFORMED AND OTHER NEOPLASTIC CELLS OBSERVED BY TIME LAPSE CIMEMATOGRAPHY. (Eng.) Paranjpe, M. S. (Natl. Cancer Inst., Bethesda, Md. 20014); Boone, C. W. *Exp. Cell Res.* 94(1):147-151; 1975.

BALB/3T3 and BALB/3T12 cells transformed by simian virus 40, TH238 cells transformed by Herpes simplex type II virus, and one of five "spontaneously" transformed neoplastic cell lines contained a significant fraction of cells that exhibited "intermittent sphering" when observed by time lapse photography. The spontaneous neoplastic transformant with the intermittent sphering property was the Sanford high tumorigenicity line from C3H mice; this line, like the Sanford low tumorigenicity line, was shown to contain C-type virus particles. The property of intermittent sphering was reflected by the tendency of stretched cells to round up and become highly refractile for some minutes as though preparing to divide, but then stretching out again for minutes or hours before undergoing sphering. The specific pattern of the frequency and duration of the sphering period was a heritable property. Occasional revertants to permanent nonsphering status were observed. Animal passage did not alter the function of intermittent sphering cells in the population. BALB/3T3 cells planted on paraffin, but not on plas-

tic substrates, also exhibited intermittent sphering, indicating that this property is related to a decrease in cell-substrate adhesivity.

1408 POLYPEPTIDES OF MAMMALIAN ONCORNAVIRUSES. III. LOCALIZATION OF p15 AND REACTIVITY WITH NATURAL ANTIBODY. (Eng.) Ihle, J. N. (Biol. Div., Oak Ridge Natl. Lab., Tenn.); Hanna, M. G., Jr.; Schäfer, W.; Hunsmann, G.; Bolognesi, D. P.; Hüper, G. *Virology* 63(1):60-67; 1975.

Localization of the polypeptide p15 in Friend murine leukemia virus was examined making particular use of recent observations that demonstrated the widespread occurrence of natural antibody to murine leukemia virus in mice. In an effort to determine whether p15 was situated on the virus envelope, its ability to interact with intact virus was analyzed by a radioimmune precipitation assay. Antiserum prepared in rabbits against p15 of Friend leukemia virua reacted with [^{3}H]leucine-labeled AKR virus. The antigen-antibody complexes formed by this reaction were isolated and shown to contain p15 after analysis by polyacrylamide gel electrophoresis. Natural antibody in mice to murine leukemia virus reacted in a similar fashion with p15, and also with the virus glycoproteins, gp71 and gp45. Further studies indicated that gp71 rather rather than p15 is principally involved in the virus-neutralization reaction; p15 antiserum was at least 20-fold less effective than gp71 antisera in neutralization of Friend leukemia virus. It is suggested that the polypeptide p15 is closely associated with the virus surface, and is one of the natural antigenic determinants of endogenous murine leukemia virus. The protein has some properties in common with membrane proteins containing hydrophobic tails in the lipid bilayer.

1409 HETEROGENEITY OF TURNOVER RATES OF 4S RNAs IN FRIEND VIRUS-INFECTED MOUSE LEUKEMIA CELLS. (Eng.) Litt, M. (Oak Ridge Lab., Oak Ridge, Tenn. 37830). *Biochem. Biophys. Res. Commun.* 66(2): 658-664; 1975.

The turnover rates of the 4S RNAs in Friend virus (FL)-infected mouse leukemia cells were studied. FL cells (GM-86, clone 745) were labeled for one generation with ^{14}C-uridine or ^{3}H-uridine, after which the ^{3}H-labeled cells were transferred to nonradioactive medium and allowed to grow exponentially for 72 hr. The low molecular weight cytoplasmic RNAs isolated from the ^{14}C- and ^{3}H-labeled cells were subjected to electrophoresis on polyacrylamide gels and cochromatographed on reverse-phase columns. The ratio of radioactivity in the 28S peak to that in the 18S peak was always 2.5, and the half-life of the 4S RNA was estimated to be about 2.5 days. The elution profile of the labeled cells after cochromatography on RPC-5 columns displayed considerable heterogeneity in the ^{3}H/^{14}C ratios. The ^{3}H/^{14}C ratios for the species eluting with the majority of the 4S RNA species spanned a range of 1.75-fold. This range represents a minimum estimate for the degree of heterogeneity of 4S RNA turnover rates. Very high ^{3}H/^{14}C ratios were also attributable to a small amount of 5S RNA. The results suggest that, during exponential growth of FL cells, different 4S RNA species are degraded at different rates.

1410 RNA METABOLISM OF MURINE LEUKEMIA VIRUS. II. ENDOGENOUS VIRUS-SPECIFIC RNA IN THE UNINFECTED BALB/c CELL LINE JLS-V9. (Eng.) Fan, H. (Tumor Virol. Lab., Salk Inst., San Diego, Calif.); Besmer, P. *J. Virol.* 15(4):836-842; 1975.

Type C virus-specific RNA sequences of BALB/c endogenous virus, previously detected in JLS-V9 cells (an uninfected BALB/c derived line) by annealing cell RNA with ^{3}H-labeled virus-specific DNA, were further characterized. Endogenous virus used in preparing the ^{3}H-labeled DNA was derived from JLS-V9 cells induced to produce virus with iododeoxyuridine (40 μg/ml). Whole cell extracts, lysed with 0.5% sodium dodecyl sulfate and 500 μg/ml proteinase K, showed two virus-specific RNA species having sedimentation rates of 38S and 27S. No 60-70S virus-specific RNA was observed. The same two species were observed in about the same ratio in RNA extracted from detergent-lysed JLS-V9 cytoplasm and in cytoplasmic RNA selected for polyadenylic acid-containing species by binding and elution from oligo(dT) cellulose. Only a small fraction of the virus-specific RNA was active as messenger RNA on polyribosomes. ^{3}H-labeled DNA prepared from endogenous N-tropic virus did not hybridize measurably with JLS-V9 RNA, indicating that the two virus-specific species are not derived from the BALB/c N-tropic endogenous virus, while DNA prepared from endogenous X-tropic virus did hybridize maximally with JLS-V9 RNA. It is thus likely that the 38S RNA is the transcription product of the whole endogenous X-tropic virus genome.

1411 A MAJOR GENETIC LOCUS AFFECTING RESISTANCE TO INFECTION WITH MURINE LEUKEMIA VIRUSES. IV. DOSE-RESPONSE RELATIONSHIPS IN *Fv-1*-SENSITIVE AND RESISTANT CELL CULTURES. (Eng.) Pincus, T. (Sloan Kettering Inst. Cancer Res., New York, N.Y.); Hartley, J. W.; Rowe, W. P. *Virology* 65(2):333-342; 1975.

The resistance to N- and B-tropic viruses controlled by the murine genetic locus *Fv-1* was studied by dose-response analysis, using indirect titration in which the number of resistant cells productively infected was assayed by infectious center plating on sensitive cell cultures. Mouse embryo (ME) cultures were prepared from 14- to 16-day-old embryos. Infectious center assay procedures were followed, and the multiplicity of infection (moi) was calculated based on virus titration and cell counts. Dose-response curves were graphed on log x log plots, and "hitness" was defined as the minimum number of interacting particles required to give a response. Three components contributed to reduced plaquing efficiency on *Fv-1* resistant cells. First, dose-response relations in *Fv-1* resistant cells showed multiple-hit kinetics; most virus strains showed 2-hit kinetics; and Gross Passage A virus gave 3-hit kinetics. Second, only a small fraction of *Fv-1*-resistant cells receiving hits became virus producers. Third, only a fraction of virus-producing cells registered as

plaques when left with resistant cells. The data identifies three factors that reduce plaquing efficiency of N- and B-tropic viruses in *Fv-1*-resistant cells: (a) "hitness factor", (b) "refractoriness" (only a fraction of cells become virus producers), and (c) virus producers. Once infection is established, virus yield from sensitive and resistant cells is similar.

1412 CHROMATOGRAPHIC SEPARATION AND ANTIGENIC ANALYSIS OF PROTEINS OF THE ONCORNAVIRUSES. V. IDENTIFICATION OF A NEW MURINE VIRAL PROTEIN, p15(E). (Eng.) Ikeda, H. (Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021); Hardy, W., Jr.; Tress, E.; Fleissner, E. *J. Virol.* 16(1):53-61; 1975.

Murine leukemia virus (MuLV) proteins were studied by various serological and other procedures; particular attention was given to the p15 protein(s). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on viruses grown in various cell types. Viral proteins were isolated by gel filtration in 6 M guanidine hydrochloride (GuHCI); immunofluorescence, immunofluorescence absorption, and immunoprecipitation and electrophoretic analysis of radiolabeled proteins precipitated by specific antisera were performed. MuLV p15(E) was visualized by SDS-PAGE, but not by gel filtration in GuHCI. It was deemed that the p15(E) polypeptide, of molecular wt 40,000, represents a protein of the actin type, which is a major host constituent and occasional contaminant. The viral specificity of p15(E) was determined serologically; anti-p15 sera precipitated p15(E), which apparently eluted in the void volume fraction of the gel filtration eluate. Differential labeling of MuLV p15(E) with single amino acids, and the analogous results obtained by [^{3}H]leucine and [^{14}C]arginine labeling, reveal that p15(E) is distinct from p15, p12, and p10. The p15(E) protein in both M-MuLV and F-MuLV viruses was specifically recognized by anti-void volume serum, supporting the conclusion that p15(E) is quantitatively precipitated in the presence of specific antisera. The results indicate that MuLV virions contain a total of seven structural proteins, while the p15 polypeptides appear to have similar molecular weights demonstrated by gel filtration. Whereas the type-specific p15 is a virion core protein, the group-specific p15(E) appears to be situated on the viral envelope.

1413 CHARACTERIZATION OF SPONTANEOUS, CHEMICAL, AND VIRAL TRANSFORMANTS OF A C3H/3T3-TYPE MOUSE CELL LINE BY TRANSPLANTATION INTO YOUNG CHICK BLASTODERMS. (Eng.) Mareel, M. (Clin. Radiother. Nuc. Med., Akademisch Ziekenhuis, De Pintelaan 135, B-9000 Ghent, Belgium); De Ridder, L.; De Brabander, M.; Vakaet, L. *J. Natl. Cancer Inst.* 54(4):923-929; 1975.

The validity of the chick blastoderm model for the detection of malignant transformations in tissue culture cells was tested. The inhibition of closure of lower germ layers by various C3H/3T3-type cells was compared with growth pattern, capacity to invade embryonic skin explants and tumorigenicity. All cells derived from C3H mouse embryos were grown as monolayers. These included mouse embryo fibroblast cells (MEF), MO, MOT, MCA, MOP, MO_4, MO_5, and MO_4Ra. To ascertain tumorigenicity, cell cultures were trypsinized, resuspended, diluted to either 5×10^6 or 5×10^3 cells/ml, and injected (0.2 ml) into female C3H mice. Tumor formation was evaluated by palpation, weight, and histologic examination after s.c. injection, or by death rate and autopsy data in i.p.-injected animals. The MEF and MO cultures grew to transparent sheets at confluency where mitotic activity decreased and were considered 'untransformed' according to growth pattern. MO cells from the 64th passage and MO_5, MOT, MCA, MO_4, MOP and MO_4Ra cells showed a transformed growth pattern. All fragments of C3H/3T3 type monolayer cultures formed distinct nodules after 24 hr incubation on chick blastoderm. Cells from MEF primary cultures always allowed repair of the defect, as did grafts from MO cultures during early passage. All other cell lines mentioned inhibited closure of the lower layer. Histologic examination of control cultures showed that embryo skin explants could be kept in organotypical culture for at least four days. MEF and MO cells showed extensive necrosis after four days. All other cells invaded the connective tissue through the epidermal wound. All cell lines except MEF and MO lines gave fibrosarcomas invading the surrounding tissue. The inhibition of lower level defect closure is a reliable, sensitive and rapid test for the detection of malignancy in tissue culture cells from any source.

1414 VIRUS-SPECIFIC DNA SEQUENCES IN MOUSE AND RAT CELLS TRANSFORMED BY THE HARVEY AND MOLONEY MURINE SARCOMA VIRUSES DETECTED BY *IN SITU* HYBRIDIZATION. (Eng.) Loni, M. C. (St. Louis Univ. Sch. Med., Mo.); Green, M. *Virology* 63(1):40-47; 1975.

The [^{3}H]DNA product of the murine sarcoma-leukemia virus [MSV(MLV)] and viral 60-70 S [^{3}H]RNA was annealed with cytological preparations of mouse and rat cells transformed by the Harvey and Moloney strains of MSV. With viral [^{3}H]DNA as cytological probe, these *in situ* hybridization measurements detected from 25 to 30 autoradiographic grains/interphase nucleus of transformed cells and 1 to 3 grains/nucleus of uninfected rat and mouse cells. With viral 60-70 S [^{3}H]RNA of lower specific radioactivity as cytological probe, 12 grains/transformed cell nucleus were detected. These findings indicate that transformation of cells with MSV(MLV) produces a several-fold increase in the content of some virus-specific DNA sequences. Virus-specific sequences in transformed mouse cells were localized in the chromocenters of interphase nuclei.

1415 MAMMARY TUMOR VIRUS PARTICLES IN THE SUBMAXILLARY GLAND, SEMINAL VESICLE, AND NONMAMMARY TUMORS OF WILD MICE. (Eng.) Rongey, R. W. (Univ. of Southern California Sch. of Medicine, Los Angeles, Calif. 90033); Abtin, A. H.; Estes, J. D.; Gardner, M. B. *J. Natl. Cancer Inst.* 54(5):1149-1156; 1975.

Type A, B, and C mammary tumor virus particles were detected in 58 adult wide mice (*Mus musculus*) trapped at three different sites in southern California. The mice were killed promptly after being trapped, and biopsy specimens were prepared for electron microscopy by fixation in 2.5% glutaraldehyde. Several spontaneous tumors arising in nonbreeding aging wild mice held in captivity were also examined electron microscopically and by the complement fixation test for murine type-C virus gs antigen. Type B particles were detected in the submaxillary glands of six of 27 pregnant wild mice; type B particles were also found in three of 24 seminal vesicles and two pulmonary adenomas. However, no type B particles occurred in the submaxillary gland of 7 nonpregnant mice or in 14 males. Within the submaxillary gland, the particles were few to moderate in number, and were found only in the lumina of the mucinous acini; they were located in seminal vesicles at the apex of the mucosal lining cells on vacuoles or in the lumina. Type A particles were detected in the submaxillary gland of two of 27 pregnant females, one of 14 males, in the seminal vesicle of two of 24 males, and in seven spontaneous nonmammary tumors, i.e. spontaneous lymphomas, pulmonary adenomas, and a hepatoma. Type C particles were also noted in many of the submaxillary glands and seminal vesicles. In wild mice, mammary tumor virus type-B particles can be detected in moderate frequency in biopsy specimens of the normal submaxillary gland (22%) and the seminal vesicle (12%). A lower frequency (60%) was found in lactating breast tissue and milk than was previously reported. The occurrence of type-B particles in the submaxillary gland of pregnant females only suggests that pregnancy stimulates type B particles production in the submaxillary gland. In addition to milk, saliva and semen as potential avenues of type-B and type-C virus transmission in wild mice must be recognized.

1416 VIRION-ASSOCIATED AND CELLULAR RNA METHYLASE ACTIVITY IN NORMAL AND NEOPLASTIC MAMMARY TISSUE FROM MAMMARY TUMOR VIRUS-INFECTED AND -UNINFECTED MICE. (Eng.) Gantt, R. (Building 37, Room 4D12, NIH, Bethesda, Md., 20014); Smith, G. H.; Julian, B. T. *Cancer Res.* 35(7):1847-1853; 1975.

The cell-free RNA methylase capacities and methylation patterns of prelactating, lactating, and neoplastic mammary tumors were comparatively studied. In addition, purified preparations of mouse mammary tumor virus (MMTV) and intracytoplasmic A particles were examined for the presence of RNA methyltransferase activity. Inbred mouse strains and sublines included BALB/c/He, BALB/cfC3H, BALB/c/CRGL, C3H/He, C3HfB/He, and C3H/StWi. Purified MMTV samples were obtained from infected milk, and the assay solutions of hydrolyzed RNA were subjected to column chromatography. When cell-free extracts were incubated with methyl-labeled S-adenosylmethionine (50 μCi) under limiting RNA conditions, differences in the extent of incorporation suggested qualitative differences in the methylase complements of the extracts. The extent of methylation did not correlate with the presence or absence of the standard MMTV(s) or low oncogenic strain MMTV(l) of MMTV. In studying two normal control tissues, the methylase activity of lactating tissue was twice that of 16 day pregnant tissue, suggesting functional changes and demonstrating the greater capacities of lactating tissue homogenates. A determination of the patterns of methylation in the four major nucleic acid bases indicated that the increased methylase activity of lactating tissue resulted from enhanced activity of all the methylases. Methylation patterns of MMTV(s) and MMTV(l) indicated that there were methylation differences, independent of the malignant state, resulting from the virus infection. RNA methylase assays in MMTV(s) virions revealed that activity is dependent on addition of both surfactant, for disruption of the virion envelope, and substrate RNA. The results suggest that the RNA methylases of mammary tumor tissue are qualitatively different from those of normal lactating mammary tissue, that no correlation exists between methylase capacity and either the malignant state or the etiological agent, and that qualitative effects are attributable to the presence of virus. These conclusions are consistent with the hypothesis that aberrant methylation of nucleic acids is a fundamental event in malignant conversion.

1417 LONG-TERM PRIMARY CULTURE OF MOUSE MAMMARY TUMOR CELLS: PRODUCTION OF VIRUS. (Eng.) Young, L. J. T. (Sch. of Medicine, Univ. of California, Davis, Calif. 95616); Cardiff, R. D.; Ashley, R. L. *J. Natl. Cancer Inst.* 54(5): 1215-1221; 1975.

The characterization of the mouse mammary tumor virus (MMTV) in long term primary cell cultures of mammary tumors, and a reassessment of the role of hormones in MMTV production and release is presented. Spontaneous mouse mammary tumors were obtained from multiparous tumor-bearing BALB/cfC3H/Crgl females, and maintained in Dulbecco's medium with fetal calf serum, insulin (10 μg/ml), hydrocortisone (10 μg/ml) and antibiotics. Isopyknic centrifugation of medium harvested from primary culture tumor cells resulted in a light-scattering band at 1.17 $\mu g/cm^3$ in sucrose, which was found to contain MMTV antigen and RNA-dependent DNA polymerase activity preferentially Mg^{++} dependent. The quantity of virus released into the medium was directly related to the initial density of the cells seeded; optimal virus production was obtained at plating density of 1×10^6 to 1.2×10^6 cells/cm^2 yielding 20-40 μg/24 hr. Detectable levels of MMTV appeared in medium by the third day after culture initiation and an initial peak was reached between the seventh and 10th day; fluctuating quantities of MMTV were released daily, and MMTV production declined with increasing age of the cell culture. Examination of the relative role of hormones with a series of deletion and addition experiments revealed that cultures initiated with insulin alone released basal levels of MMTV. Substitution of hydrocortisone for insulin resulted in release of three times the basal levels of MMTV, while initiation and maintenance of cultures with both insulin and hydrocortisone yielded eight times the basal MMTV levels. Reversibility of such effects was noted. The experiments indicate that cell density and the hormonal milieu are the critical factors on virus yield, and that hydrocortisone

has a primary effect on MMTV release, whereas insulin acts synergistically with hydrocortisone to maximize MMTV production and release.

1418 PRODUCTION OF MOUSE MAMMARY TUMOR VIRUS BY CULTURED CELLS IN THE ABSENCE AND PRESENCE OF HORMONES: ASSAY BY MOLECULAR HYBRIDIZATION. (Eng.) Ringold, G. (Dep. Microbiol., Univ. California, San Francisco); Lasfargues, E. Y.; Bishop, J. M.; Varmus, H. E. *Virology* 65(1):135-147; 1975.

The regulation of mouse mammary tumor virus (MMTV) gene expression was studied by measuring the production of virus and the transcription of viral genes in cultured cells. MMTV from the milk of RIII mice was used. A new assay based on the hybridization of labeled virus-specific DNA (cDNA) with RNA extracted from virus released into the growth medium was used to measure virus production. Levels of virus-specific RNA in various cells were measured by annealing MMTV cDNA with cell RNA. In cell lines and primary explants derived from mammary tumors of several strains of mice the amount of virus production correlates with the level of virus-specific RNA. Experiments with dexamethasone (a synthetic glucocorticoid) support the suggestion that transcriptional controls are of primary importance in regulating the production of MMTV in these cells. Cells treated with the hormone show parallel increases in virus production and intracellular virus-specific RNA. In contrast, a lymphoma cell line (S49) derived from a lymphoma induced by mineral oil in a BALB/c mouse contains large quantities of MMTV-specific RNA yet produces extremely low levels of virus. In these cells, mechanisms other than transcriptional controls may be important in regulating virus production.

1419 DISTINCTION BETWEEN MALIGNANT L CELLS AND NORMAL MOUSE FIBROBLASTS BY ROSETTE FORMATION WITH SHEEP RED BLOOD CELLS. (Eng.) Hoch, J. A. (Scripps Clin. Res. Found., La Jolla, Calif.); Dierich, M. P.; Pellegrino, M. A.; Ferrone, S.; Reisfeld, R. A. *J. Immunol.* 114(5):1638-1640; 1975.

The reactivity of cell surface markers was investigated by rosette formation. The lines included malignant L-929, 1R, CL-1D, and B82 from C3H mice; 1T-22 from BALB/c mice, primary kidney from B10BR, C57BL, and C3H mice; primary tumor from C3H mice; BHK-2 from hamster kidney; Pv-BHK from polyoma transformed BHK-2; and AD from human fetal lung. All five lines from malignant mouse L cells were capable of forming rosettes with sheep RBC (SRBC). The 1T-22 cell line did not rosette with SRBC and the primary explants from normal C3H kidney did not rosette SRBC; all other explants were also negative. Cells with potent rosetting capacity were frequently found adjacent to fibroblasts with no adherent SRBC. Preformed rosettes were sensitive to 0.25% trypsin. Rosette formation is also time-dependent because no rosettes formed after one hr of incubation, whereas maximal rosetting occurred after 3-4 hr. Hybrid cells lines (1R x 1T-22) failed to form rosettes with SRBC, suggesting that 1T genetic material suppresses the expression of rosetting with SRBC. Rosette formation by L cells appears to represent the expression of a recessive trait.

1420 ENDOGENOUS ONCORNAVIRUSES IN CHEMICALLY INDUCED TRANSFORMATION. I. TRANSFORMATION INDEPENDENT OF VIRUS PRODUCTION. (Eng.) Rapp, U. R. (McArdle Lab. Cancer Res., Univ. Wisconsin, Madison); Nowinski, R. C.; Reznikoff, C. A.; Heidelberger, C. *Virology* 65(2):392-409; 1975.

The state of endogenous oncorna viruses in C3H/10T1/2 cells was characterized by determining the production of infectious virus or viral antigens. The inducibility of endogenous viral genomes was determined by treating the cells with 5-iododeoxyuridine (IUdR). Mouse embryo fibroblasts (MEF) and cell lines from C3H and AKR mice were maintained *in vitro*. Spontaneously transformed C3H/10T1/2 cells were analyzed for tumorigenicity and endogenous oncornaviruses. Gross Passage A virus and MuLV(MSV), Moloney strain, were grown in the cells. XC plaque assays were made five days after infecting III 6 A cells. Rat-tropic nontransforming viruses were assayed by focus induction on the nonproductively murine sarcoma virus-transformed NRK-1a cell line. Determination of oncornaviruses were made by infectious center assay. Immunofluorescence tests were used to detect murine leukemia virus, group-specific-antigen, and the murine leukemia virus cell surface G_L and G_T. DNA polymerase assays were also made. The media from all chemically transformed cell lines tested were found to be consistently virus-negative. All tests for oncornaviruses or oncornavirus-associated antigens in C3H/10T1/2 were negative. Oncornaviruses induced from IUdR-treated cells were capable of transforming normal C3H/10T1/2 fibroblasts in culture. Genetic information responsible for the transformation of C3H cells was not available for rescue by an exogenous oncornavirus. Genes responsible for transformation are not related to a virus-coded transformation gene.

1421 INDUCTION OF A CARCINOGENIC ONCORNAVIRUS IN C57BL/6 MOUSE EMBRYO CELLS BY 5-IODODEOXYURIDINE. *(Eng.)* Lazar, A. (Hebrew Univ.-Hadassah Medical Sch., Jerusalem, Israel); Schlesinger, M.; Horowitz, A. T.; Heller, E. *Nature* 255 (5510):648-650; 1975.

The induction of an oncornavirus followed by the transformation of an epitheloid line of mouse embryo cells to carcinoma cells is described. Cell cultures were initiated from pooled whole embryos of individual C57BL/6 mice at 12-14 days gestation. Of 17 cultures isolated, five developed into cell lines; the epitheloid M6 line was used in subsequent experiments. M6 cells of passage 31 were used for induction by iododeoxyuridine (IdU) plus dexamethasone. The number of syncytium-forming cells (SFC) in the first and second passages was 0 and 0.001%; thereafter, the SFC rapidly increased to 75%, which declined to levels below 1% after nine further passages. In repeat experiments using M6 cells at passages 37 and 45, similar kinetics of syncytium production with some variation in the

time of appearance was noted, suggesting that M6 cells were activated by IdU to produce an oncornavirus (BVI). Labeling with ^{3}H-uridine indicated that BVI is a particle with a density characteristic of oncornaviruses, and is released from cells. Electron microscopic examination of induced M6 cells revealed C-type particles in the intercellular spaces, plus two stages of budding particles. Comparitive studies on M6 virus-positive and -negative cells showed that only the infected cells were competent to replicate both BVI and RadLV viruses and to yield syncytia, while M6 virus-negative cells produced 30 times more colonies as M6 cells; this indicates that M6 virus-negative cells are transformed nonproducer cells. The data indicate that the induced cells and the infected cells were transformed, and suggest that some viral function(s) is needed for initiation of cell transformation.

1422 IMMUNOLOGICAL PROPERTIES OF AVIAN ONCORNAVIRUS POLYPEPTIDES. (Eng.) Bolognesi, D. P. (Duke Univ. Med. Cent., Durham, N. C.); Ishizaki, R.; Hüper, G.; Vanaman, T. C.; Smith, R. E. *Virology* 64(2):349-357; 1975.

The serological interrelationships among the structural components of viruses originating from several distinct chicken leukemic sarcoma virus (ChiLSV) subgroups were investigated. The major polypeptides and glycoproteins were isolated by gel filtration in guanidine hydrochloride. Antibody titers were obtained by complement fixation and radioimmunoassay. All the antisera reacted strongly with homologous polypeptides in both tests; only some minor reactivity was detected with heterologous polypeptides. Radioimmunoassay was used to test for cross-reacting determinants. Anti-p15 reacted to both p15 and p27 while anti-p27 reacted only to p27, indicating no contamination; complete competition was obtained with 10 ng of either unlabeled p15 or p27; p15 antisera was thus contaminated by anti-p27. Analyses of polyproteins from avian myeloblastosis virus (AMV) (p27, p15, p12) and from Prague strain Rous sarcoma virus subunit PR-RSV-C indicated that these components were serologically similar to AMV since the PR-RSV polypeptides competed as effectively as the AMV polypeptides for the respective AMV antisera. This was not the case for p19, indicating that p19 peptides of AMV and PR-RSV viruses are not serologically identical. These results confirm that the major internal virus proteins possess group-specific determinants and specific reactivity to surface glycoproteins associated with internal (p19) polypeptides. It is also concluded that no significant serological relatedness exists among the four virions of ChiLSV.

1423 BIOCHEMICAL CHARACTERIZATION OF TUMOR-SPECIFIC CELL SURFACE ANTIGENS ON AVIAN ONCORNAVIRUS TRANSFORMED CELLS. (Eng.) Rohrschneider, L. R. (Institut für Virologie, Bereich Humanmedizin, Justus Liebig-Universität, 63 Giessen, Frankfurter Strasse 107, West Germany); Kurth, R.; Bauer, H. *Virology* 66(2):481-491; 1975.

A sensitive indirect immune precipitation method coupled with polyacrylamide-gel electrophoresis was used to detect new antigens from avian oncornavirus-transformed chicken embryo cells (CEC). A total of four antigens were recognized by this method. Two of these antigens appeared to be identical to the gp85 and gp37 components of the virus envelope. The other two antigens were distinct from the viral envelope antigens on the basis of both size and antigenicity, and were group-specific for the avian oncornavirus system. The larger nonvirion antigen was detectable on transformed CEC but was absent from normal or productively infected nontransformed CEC and therefore was clearly tumor-specific. Because of the weak detectability of the smaller nonvirion antigen, its tumor specificity could not be decided. The tumor-specific antigen was a glycoprotein of 100,000-dalton apparent molecular wt, whereas the smaller nonvirion antigen was 32,000 daltons and probably also a glycoprotein. The 100,000-dalton tumor-specific antigen was found on the cell surface and therefore represented a tumor-specific cell surface antigen (TSSA). Further experiments are indicated to determine if the TSSAs found are capable of immunizing animals against tumor transplants.

1424 STRUCTURAL POLYPEPTIDES OF RABBIT, BOVINE, AND HUMAN PAPILLOMAVIRUSES. *(Eng.)* Favre, M. (Unite de Biochimie Enzymologie, Institut Gustave-Roussy, 94800 Villejuif, France); Breitburd, F.; Croissant, O.; Orth, G. *J. Virol.* 15(5):1239-1247; 1975.

The number and apparent molecular weight of the structural polypeptides of the Shope rabbit papilloma virus (RPV), bovine papilloma virus (BPV), and human papilloma virus were estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Up to 10 polypeptides were detected in highly purified BPV and HPV full particles, a close homology being found between the polypeptide compositions of these two viruses. Purified RPV virious gave a similar polypeptide pattern. The primary components of the three viruses were a major polypeptide (VP1), with a mol wt of about 54,000, and three smaller polypeptides (VP8, 9, and 10), with molecular weights of about 16,500, 51,500, and 12,500, respectively. Up to seven polypeptides, including the major polypeptide, were detected in empty BPV capsids, and six polypeptides, including the major polypeptide, were detected in empty HPV capsids; VP8, 9, and 10 were never detected in empty capsids. When BPV virions were disrupted with alkaline buffer, the six lower-molecular weight polypeptides (VP5-10) remained associated with the viral DNA. Comparison of the polypeptide compositions of BVP and polyoma virus, another papovavirus, showed that the number of components and the molecular weight of the major polypeptide differed between the two viruses. However, the three main DNA-associated polypeptides of BVP (VP8, 9, and 10) and the three histone-like components of polyoma virus (VP4, 5, and 6) had identical apparent molecular weights. The data suggest that BPV components VP5-10 are internal components of the virions and that components VP1-4 may be external components. The data further suggest that some of the minor components of the papil-

loma viruses may be proteolytic degradation products or cell protein contaminants.

1425 VIRION POLYPEPTIDE COMPOSITION OF THE HUMAN PAPOVAVIRUS BK: COMPARISON WITH SIMIAN VIRUS 40 AND POLYOMA VIRUS. (Eng.) Wright, P. J. (Univ. Illinois Med. Cent., Chicago); Di Mayorca, G. *J. Virol.* 15(4):828-835; 1975.

The polypeptide composition ot BK virus was compared with two other papovaviruses, SV40 and polyoma. Disrupted virions were subjected to co-electrophoresis on 10% polyacrylamide gels containing sodium dodecyl sulfate using a continuous gel system. BK virus was labeled with ^{3}H-labeled reconstituted protein hydrolysate (10 μCi/ml) or (^{3}H)lysine (0.2 μCi/ml), and the other two viruses were labeled with ^{14}C-labeled reconstituted protein hydrolysate (1 μCi/ml). The major polypeptide (VP1) of BK virus constituted about 73% of the total capsid protein and had a molecular weight of 39,000. It was smaller than the VP1 of SV40 and polyoma virus. The other BK virus polypeptides were similar in molecular weight to those of SV40. A comparison of the BK virus proteins and SV40 proteins iodinated with chloramine T before and after dialysis against 0.15 M carbonate-bicarbonate buffer, pH 10.5 at 5 C for 16 hr, revealed differences in the number and distribution of tyrosine units available for iodination in the two viruses. Using an ion exchange column, SV40 tryptic peptides of viral proteins VP1, VP3, VP4, and VP5 were compared with those of the same proteins of BK virus. Among the 19 peptides of VP1 resolved, only two were common to both viruses. No peptides were common to VP3 of both viruses. There are thus major differences in the primary capsid protein structures of the two viruses. Six of 15 resolved peptides of VP4 and VP5, however, were common to both viruses. This similarity is consistent with the claim that VP4 and VP5 may be histones, coded for by the cell genome.

1426 POLYOMA VIRUS-TRANSFORMED RAT CELL LINES INDUCIBLE FOR VIRAL CAPSID ANTIGEN SYNTHESIS. (Eng.) Fogel, M. (Dep. Genet., Weizmann Inst. Sci., Rehovot, Israel). *Virology* 65(2):446-454; 1975.

Polyoma virus (PV)-transformed hamster, rat and mouse cell lines were established free of polyoma virus capsid (V)-antigen and infectious virus in order to determine their inducibility to mitomycin C. Samples containing 10^{5} cells in 0.1 ml of medium were incubated with anti-PV serum sufficient to neutralize 2×10^{5} plaque forming units (PFU) of virus. Anti-V-antigen serum, conjugated with fluorescein, stained V-antigen when tested on PV-infected mouse embryo cells. Cultures were treated with mitomycin C at concentrations of 0.25, 0.5 and 1.0 μg/ml for one hr at 37 C. Induction of PV or V-antigen synthesis was detected in four lines of rat origin. V-antigen-containing cells in the RPA and RPB cultures were preincubated at 40 C; the RPB line produced low yields at both temperatures. Following mitomycin C treatment, V-antigen could be detected in six RPA and 14 RPB clones. RPB clones yielded small quantities of virus. The author suggests that the lack of virus production in the V-antigen-containing RPA and in most RPB cells is due to a deficiency of the viral genome. A temperature-sensitive factor is involved in the process of V-antigen induction in the RPA and RPB lines.

1427 NONHISTONE VIRION PROTEINS OF POLYOMA: CHARACTERISATION OF THE PARTICLE PROTEINS BY TRYPTIC PEPTIDE ANALYSIS BY USE OF ION-EXCHANGE COLUMNS. (Eng.) Hewick, R. M. (Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London, England); Fried, M.; Waterfield, M. D. *Virology* 66(2):408-419; 1975.

The primary structure of various differentially labeled ([^{14}C]- or [^{3}H]lysine plus arginine) polypeptides were compared by an analysis of their tryptic peptides by use of ion-exchange and paper chromatography. Highly purified polyoma virus particles contain four nonhistone polypeptides. Their approximate molecular weights are 86,000 (VP0), 48,000 (VP1), 35,000 (VP2), and 23,000 (VP3). The results indicate that VP0 and VP1 have identical peptide maps and that VP0 is probably a dimer of VP1. All of the tryptic peptides derived from VP3 were also detected in the tryptic digest of VP2. Additional peptides that were found in the VP2 digest and not in the VP3 digest may account for the difference in molecular weight of these two polypeptides. Thus VP3 appears to be derived from VP2. Whether this is of biological significance is not known. VP1 shares very few, if any, tryptic peptides with VP2 and VP3. These results are consistent with there being two polyoma genes coding for the virion proteins of either VP1 or VP2.

1428 TRANSCRIPTION OF THE REPETITIVE DNA SEQUENCES IN POLYOMA-TRANSFORMED AND NONTRANSFORMED MOUSE CELLS IN CULTURE. (Eng.) Grady, L. J. (Div. Lab. Res., New York State Dep. Health, Albany); Campbell, W. P. *Cancer Res.* 35(6):1559-1562; 1975.

RNA-DNA saturation hybridization experiments were used to estimate the extent of transcription of repetitive DNA sequences in transformed and nontransformed mouse cells in culture. Nontransformed AL/N mouse cells, and a polyoma-transformed derivative, PY AL/N clone 3, were used. Measurements were made with RNA from nontransformed cells in both subconfluent and confluent stages of growth, transformed cells in normal growth medium, and transformed cells grown in medium containing 5-bromodeoxyuridine, 3 μg/ml, (to reduce their tumorigenic potential). No differences were observed in the amount of repetitive DNA transcribed or in the families of sequences expressed, except in transformed cells grown in the presence of 5-bromodeoxyuridine, in which case the extent of transcription was reduced. The 3.5% of the DNA that formed hybrids, if corrected for the 10% of the genome removed by the initial fractionation, represents only 3.1% of the genome. However, since only repetitive DNA (20% of the DNA used) was

able to form hybrids, it can be considered equivalent to 17.5% of the repetitive sequences.

1429 FATE OF POLYOMA FORM I DNA DURING REPLICATION. (Eng.) Roman, A. (Univ. Oregon Medical Sch., Portland, Oreg. 97201); Dulbecco, R. *J. Virol.* 16(1):70-74; 1975.

The fate of polyoma form I DNA generated during replication was investigated in resting BALB-3T3 cells. The cells were plated and exposed to polyoma virus (100 plaque forming units/cell) for one hour. Post-infection cells were labeled for 20 min with 50-100 μCi [^{3}H]thymidine, and the viral DNA was extracted. Form I DNA was separated from form II and replicative intermediate (RI) DNA by sucrose gradient centrifugation. Label in form I increased during the first 20 min of chase, and then remained constant. The kinetics of re-entry of form I into replication showed that 89% of the [^{3}H]thymidine was associated with form I DNA. Two main characteristics of the re-entry of form I molecules into replication were: (1) re-entry was extensive because it involved half the labeled DNA, and (2) molecules generated during a short time (20 min) returned to a replicating pool, suggesting that the re-entry process is random. The fate of progeny form I DNA is determined by the availability of initiation and maturation protein. For the replicating pool to expand, RI formation must be accelerated, and the accummulation of form I DNA indicates that a constant number undergoes replication.

1430 5'-TERMINAL m^{7}G(5')ppp(5')G^{m}p *IN VIVO:* IDENTIFICATION IN REOVIRUS GENOME RNA. (Eng.) Furuichi, Y. (Roche Inst. Mol. Biol., Nutley, N. J.); Muthukrishnan, S.; Shatkin, A. J. *Proc. Natl. Acad. Sci. USA* 72(2):742-745; 1975.

The presence of unusual 5' terminal structures in *in vitro*-produced viral messenger RNAs (mRNA) was studied. Reovirus type 3 Dearing strain was purified from infected mouse L cells, and the double-stranded RNA was isolated. Genome RNA uniformly labeled with ^{32}P was mixed with ^{3}H-labeled mRNA synthesized *in vitro* in the presence of *S*-adenosyl-L-[*methyl*-^{3}H]methionine. The double-labeled mixture was digested with RNase T_2 and analyzed by chromatography on *O*-(diethylaminoethyl) cellulose; the ^{32}P-labeled material was apparently heterogeneous and did not elute coincidentally with the ^{3}H-labeled 5' termini. ^{3}H-labeled and ^{32}P-containing structures that co-eluted also migrated together when analyzed by high voltage paper electrophoresis. *Penicillium* nuclease digestion revealed that 60% of the ^{32}P was resistant to hydrolysis and migrated with the ^{3}H-labeled m^{7}GpppG^m. Alternate hydrolysis and reanalysis confirmed the finding that the 5' termini from ^{32}P-labeled genome RNA and ^{3}H-labeled mRNA were identical. A direct test of the number of blocked, terminal phosphates in reovirus mRNA demonstrated the presence of three blocked phosphates at the 5' end of reovirus mRNA and in the identical 5' terminus of the plus strand of viral genome RNA. In addition, the 5' linkage of m^{7}G was demonstrated in reovirus mRNA and in genome RNA. Both the mRNA and the corresponding strand of genome RNA thus contained m^{7}G(5')ppp(5')G^{m}pCp, indicating that infected cells synthesized viral RNA with blocked, methylated 5' termini.

1431 STRUCTURAL PROTEINS OF MAMMALIAN RNA TUMOR VIRUSES: RELATEDNESS OF THE INTERSPECIES ANTIGENIC DETERMINANTS OF THE MAJOR INTERNAL PROTEIN. (Eng.) Strand, M. (Albert Einstein Coll. Med., Bronx, N. Y.); August, J. T. *J. Virol.* 15(6):1332-1341; 1975.

The relatedness of antigenic determinants of purified major core proteins of the murine, feline, RD 114/baboon, and woolly monkey/gibbon ape groups of RNA tumor viruses was examined by competition radioimmunoassay using ^{125}I-labeled antigen. In assay systems of a homologous antigen and antiserum, high affinity competition for binding to all of the antibodies was observed only with the homologous unlabeled protein; the core proteins of other groups of viruses showed only low affinity binding of a small fraction of antibodies, presumably those reactive with the interspecies determinants, at concentrations of competing protein 10-100-fold greater than that of the labeled antigen. The cross-reactive (interspecies) antigens of every two viruses were selectively examined by precipitating the purified ^{125}I-labeled protein with antiserum against each of the other proteins. The extent to which these shared determinants were common to the other viruses was then tested. Classes of interspecies determinants common to two of the groups of viruses, to three, and to all four were distinguished. These classes were not necessarily distinct, however, as there were differences in the affinity of cross-reactive proteins for antibody binding, as demonstrated by the experiments with ^{125}I-labeled RD 114 p28. These studies suggest that the murine and feline viruses are closely related as they contain cross-reactive antigenic determinants not shared with the other viruses, that the feline virus is more closely related to the woolly monkey virus than to RD 114 and woolly monkey viruses retain interspecies determinants shared relatively equally with each of the other viruses.

1432 QUANTITATIVE NUCLEOTIDE SEQUENCE RELATIONSHIPS OF MAMMALIAN RNA TUMOR VIRUSES. (Eng.) East, J. L. (Univ. Texas Syst. Cancer Cent. M. D. Anderson Hosp. Tumor Inst., Houston); Knesek, J. E.; Chan, J. C.; Dmochowski, L. *J. Virol.* 15(6): 1396-1408; 1975.

A molecular hybridization technique was used to measure quantitatively the nucleotide sequence relationships of three selected mammalian RNA tumor groups (murine, simian, and feline viruses). Reciprocal cross-hybridization tests were done in which a given ^{3}H-labeled, viral genomic RNA species was annealed with an excess of unlabeled, complementary DNA product synthesized in endogenously instructed reverse transcriptase reactions. Hybrid formation was measured with pancreatic RNase A. The results of reciprocal cross-hybridization testing showed that the murine viruses consist of four distinctly related subgroups: the Friend leukemia virus/Rauscher leukemia virus subgroup, the Gross leukemia virus sub-

group, the Moloney sarcoma virus subgroup, and the Kirsten sarcoma virus subgroup. Simian sarcoma virus, the only simian virus examined, appeared to share limited interspecies sequence relationships with members of the other virus groups and in particular with Kirsten sarcoma virus. Of the two members of the feline virus group tested, Rickard feline sarcoma virus and RD-114, each was placed in a separate, unrelated subgroup. Rickard feline sarcoma virus exhibited limited sequence relatedness with members of the other virus groups, whereas RD-114 exhibited none. It is concluded that genetically distinct nucleotide sequence relationships exist within a given mammalian RNA tumor virus group, and perhaps between virus groups.

1433 RECOMBINATION BETWEEN ENDOGENOUS AND EXOGENOUS RNA TUMOR VIRUS GENES AS ANALYZED BY NUCLEIC ACID HYBRIDIZATION. (Eng.) Hayward, W. S. (Rockefeller Univ., New York, N. Y.); Hanafusa, H. *J. Virol.* 15(6):1367-1377; 1975.

The nucleic acids of Rous-associated virus 60 (RAV-60) were analyzed for sequence homologies with the viral nucleic acids contained in the uninfected cell and with those of RAV-2, the exogenous virus used for the preparation of this particular RAV-60 isolate. In addition, these nucleic acids were compared with those of RAV-0, an endogenous virus spontaneously released from line 100 chicken cells. Sequence differences among the nucleic acids of RAV-0, RAV-2, and RAV-60 were readily detectable by nucleic acid hybridization techniques utilizing either ^{3}H-labeled complementary DNA or ^{3}H-RNA as probes. Based on the pattern of hybridization obtained with the nucleic acids of these viruses and on the melting profiles of the various hybrid combinations, RAV-60 appears to be intermediate between RAV-0 and RAV-2 in its genetic composition. Of the three viruses tested, RAV-0 apparently had the greatest sequence homology with the viral nucleic acids of the uninfected cell. Hybridization between RAV-60 ^{3}H-labeled complementary DNA and either DNA or RNA from the uninfected cell indicates that RAV-60 contains some nucleic acid sequences which are not present in the cell. In addition, some RAV-60 sequences which hybridize with the cell nucleic acid contain significant amounts of mismatching, as indicated by the lower thermal stability of these hybrid duplexes. Hybrid formation between these partially homologous sequences was excluded under stringent annealing conditions. The data indicate that RAV-60 is a recombinant between exogenous and endogenous viral genes.

1434 RNA TUMOR VIRUS SPECIFIC SEQUENCES IN NUCLEAR DNA OF SEVERAL AVIAN SPECIES. (Eng.) Tereba, A. (Sch. Med., Univ. South. California, Los Angeles); Skoog, L.; Vogt, P. K. *Virology* 65(2):524-534; 1975.

The nuclear DNA of normal cells from several avian species was reexamined for the presence of sequences related to the endogenous Rous-associated virus type 0 and to exogenous Rous sarcoma virus. Using DNA-DNA hybridization, nucleic acid sequences complementary to the endogenous virus were detected in nuclear DNA from five different avian species. The Rous-associated virus type 0 probe hybridized almost completely to DNA from helper factor positive and negative chicken cells. DNA from quail, duck, pigeon and pheasant cells contained nucleotide sequences complementary to a varying degree (5-50%) to the Rous-associated virus type 0 genome. Rous sarcoma virus-related sequences were also detected in all avian species, except pigeon. It is likely that most, if not all, species of fowl contain an endogenous RNA tumor virus related at least partially to the endogenous chicken RNA tumor virus, i.e., Rous-associated virus type 0. The possibility that the presence of viral-related sequences in cellular DNA may be required for a successful and correct integration of RNA tumor viruses is discussed.

1435 DIFFERENTIAL RESPONSE TO CYTOCHALASIN B AMONG CELLS TRANSFORMED BY DNA AND RNA TUMOR VIRUSES. (Eng.) O'Neill, F. J. (Univ. Utah Coll. Medicine, Salt Lake City, Utah 84132); Miller, T. H.; Hoen, J.; Stradley, B.; Devlahovich, V. *J. Natl. Cancer Inst.* 55(4):951-955; 1975.

A study was undertaken to determine the responsiveness to cytochalasin B (CB) of cells transformed by RNA and DNA tumor viruses. Mouse, hamster, rat, and chicken embryo fibroblasts, and normal rat and human embryonic kidney cells were transformed by the following viruses: simian virus 40, adenovirus type 7, Kirsten mouse sarcoma virus (Ki-MuSV), Moloney mouse sarcoma virus, and Rous sarcoma virus. All cultures of transformed cells grew to high concentration densities. Normal and transformed cells were treated with CB at concentrations preventing cytoplasmic cleavage (1-3 μg/ml in culture medium for seven days). Cells altered by DNA tumor viruses responded to CB with numerous nuclear divisions resulting in highly multinucleated cells. All but one line of cells transformed by RNA tumor viruses responded to CB with usually only one and occasionally two nuclear divisions. Only binucleated cells were formed. One clone of CB-treated BALB/c mouse embryo fibroblasts transformed by Ki-MuSV showed numerous cells with 4 and 5 nuclei. However, in contrast to cells transformed by DNA viruses, few cells had seven or more nuclei. These results suggest that, in the presence of CB, cells transformed by DNA tumor viruses show uncontrolled nuclear division, whereas cells transformed by RNA tumor viruses show controlled nuclear division.

1436 INHIBITION OF VIRUS-INDUCED CELL TRANSFORMATION BY SYNTHETIC ANALOGUES OF S-ADENOSYL HOMOCYSTEINE. (Eng.) Robert-Gero, M. (Institut de Chimie des Substances Naturelles, CNRS, 91190 GIF SUR YVETTE, France); Lawrence, F.; Farrugia, G.; Berneman, A.; Blanchard, P.; Vigier, P.; Lederer, E. *Biochem. Biophys. Res. Commun.* 65(4):1242-1249; 1975.

The effects of S-adenosyl-homocysteine (SAH) and its structural analogs, particularly 5'-deoxy-5'-S-isobutyl-adenosine (SIBA), on cell transformation by

Rous sarcoma virus (RSV) were studied. Secondary cultures of chick embryo fibroblasts (CEF) were treated with SIBA, SAH, or the other analogs at various intervals before and after RSV infection. Determinations were made of the number of foci of transformed cells, virus production, transfer RNA methylase activity, protein concentration, and macromolecular synthesis in the cultures. SAH and all 11 analogs tested strongly inhibited focus formation, but most also had cytotoxic effects on normal cells. Neither SIBA or SAH was cytotoxic for normal CEF. SIBA (0.5-1 mM) strongly inhibited oncogenic cell transformation when added up to four days after infection, while SAH had no inhibitory effect on focus formation. SIBA had no inhibitory effect on virus production when the cells were treated 24 hr before infection, but virus production was strongly inhibited when SIBA was present for at least 24 hr immediately after infection. SIBA was a weak inhibitor of transfer RNA transmethylases in normal and transformed cells and *in vitro* in cell extracts from normal and transformed cells; the inhibition of enzymes by SIBA was competitive with respect to S-adenosylmethionine. When normal cells were exposed to 1 mM SIBA for 24 hr, the synthesis of proteins, RNA, and DNA was inhibited, but the inhibition was reversible. In contrast, the inhibition was irreversible in RSV-infected cells. The results suggest that SIBA or its analogs might become valuable chemotherapeutic agents.

1437 STUDIES ON THE SMALL RNAs OF ROUS SARCOMA VIRUS. (Eng.) Sawyer, R. C. (Univ. Wisconsin-Madison). *Diss. Abstr. Int. B* 35(11):5294-5295; 1975.

The individual 4S RNA species of Rous sarcoma virus (RSV) were characterized. Two-dimensional polyacrylamide gel electrophoresis was used to fractionate the small RNAs. Spot 1 RNA was the most abundant, and it was dissociated from the 70S RNA at 63 C. Uninfected cells were used to investigate the origin of spot 1 RNA in 2-dimensional gel mobility and RNase fingerprint experiments. The complete nucleotide sequence of spot 1 RNA was determined and is presented. The 4S RNA species produced patterns characteristic of transfer RNA. Spot 1 RNA serves as the major primer RNA for *in vitro* RSV DNA synthesis; it is a cell-coded RNA or a nonrandom subset of host cell tRNA.

1438 EFFECTS OF GLUCOSE STARVATION ON NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED CHICK CELLS. (Eng.) Venuta, S. (I. Cattedra di Chimica Biologica, II Facoltá di Medicine e Chirurgia, Universitá di Napoli, Napoli, Italy); Rubin, H. *J. Natl. Cancer Inst.* 54(2):395-400; 1975.

Transformed cells are known to utilize glucose at a greater rate than normal cells. The effect of glucose starvation was tested on glucose and thymidine uptake and incorporation in normal chick embryo fibroblasts and those transformed by Rous sarcoma virus (RSV). The uptake of a metabolizable glucose analog 2-deoxy-D-glucose (2-dGlc) and the uptake and incorporation of thymidine (^{3}H-TDR) were measured in resting normal fibroblasts suspended in both glucose-free and glucose-containing media. ^{3}H-2 dGlc uptake increased about tenfold in the glucose-starved cells, but the incorporation of ^{3}H-TDR was unaffected. Similar results were obtained when a non-metabolizable glucose analog, 3-O-methylglucose (3-MeGlc) uptake was studied in the resting fibroblasts. Growing fibroblasts showed a twofold increase in glucose uptake when starved for glucose. Starvation showed no significant effect however, on either sugar transport or thymidine incorporation in transformed cells. A decrease in medium glucose concentration is thus not the cause of increased glucose transport in transformed cells. It is suggested that glucose or one of its metabolic products regulates hexose uptake by normal fibroblasts. This regulation is apparently not seen in virus-transformed cells.

1439 SIMIAN VIRUS 40 DNA SEGMENT OF THE ADENOVIRUS 7-SIMIAN VIRUS 40 HYBRID, E46$^+$, AND ITS TRANSCRIPTION DURING PERMISSIVE INFECTION OF MONKEY KIDNEY CELLS. *(Eng.)* Lebowitz, P. (Yale Univ. Sch. Med., New Haven, Conn.); Khoury, G. *J. Virol.* 15(5):1214-1221; 1975.

Nucleic acid hybridization was used to study the span of the simian virus 40 (SV40) DNA segment in the adenovirus 7-SV40 hybrid, E46$^+$, and the extent of its transcription in lytically infected monkey kidney cells. The SV40 segment of E46$^+$ was found to comprise about 62% of the SV40 genome; it originates in the proximal region of Hin-G (the G fragment derived by cleavage of intact SV40 DNA with *Haemophilus influenzae* restriction endonuclease), extends sequentially through approximately 80% of this fragment, all of fragments Hin-B, -I, -H, and -A, and terminates about 70% of the distance through Hin-C. During the E46$^+$ lytic infection of permissive cells, most of the stable cytoplasmic SV40-specific RNA was transcribed from the minus (E) strand of the fragments Hin-A, -H, -I, and -B (the early template region). Transcripts of the minus strand of the Hin-G and -C fragments were detected in much lower concentrations, especially in the total lytic cellular RNA, and RNA complementary to the plus (L) strand was not detected at all. The transcriptional pattern of the SV40 segment with E46$^+$ is similar to that in a number of transformed cell lines and in some respects is similar to the transcriptional pattern in a series of nondefective adenovirus 2-SV40 hybrid viruses. The data suggest a common transcriptional mechanism for integrated SV40 DNA.

1440 PROTEINS IN INTRACELLULAR SIMIAN VIRUS 40 NUCLEOPROTEIN COMPLEXES: COMPARISON WITH SIMIAN VIRUS 40 CORE PROTEINS. (Eng.) Meinke, W. (Scripps Clin. Res. Found., La Jolla, Calif.); Hall, M. R.; Goldstein, D. A. *J. Virol.* 15(3):439-448; 1975.

Intracellular nucleoprotein complexes containing simian virus 40 (SV40) supercoiled DNA were purified from cell lysates by chromatography on hydroxyapatite columns followed by velocity sedimentation through sucrose gradients. The major protein com-

ponents from purified complexes were identified as histone-like proteins. When analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, complex proteins comigrated with viral core polypeptides VP4, VP5, VP6, and VP7. [^{3}H]tryptophan was not detected in polypeptides from intracellular complexes or in the histone components from purified SV40 virus. However, a large amount of [^{3}H]tryptophan was found in the viral polypeptide VP3 relative to that incorporated into the capsid polypeptides VP1 and VP2. Intracellular complexes contain 30 to 40% more protein than viral cores prepared by alkali dissociation of intact virus; but when complexes were exposed to the same alkaline conditions, protein also was removed from complexes and they subsequently co-sedimented with and had the same buoyant density as viral cores. The composition and physical similarities of nucleoprotein complex and viral cores indicate that complexes may have a role in the assembly of virions.

1441 SIMIAN VIRUS 40-RELATED ANTIGENS IN THREE HUMAN MENINGIOMAS WITH DEFINED CHROMOSOME LOSS. (Eng.) Weiss, A. F. (Max-Planck-Institut für Psychiatrie, 8 München 40, Kraepelinstr. 2, Germany); Portmann, R.; Fischer, H.; Simon, J.; Zang, K. D. *Proc. Natl. Acad. Sci. USA* 72(2):609-613; 1975.

The possible presence of simian virus 40 (SV40)-specific antigens in human meningioma cells was investigated. Meningioma biopsy material was obtained from seven patients, six of whom had primary tumors, and was trypsinized and cultured. Primary human fibroblast, human glioblastoma, MA 134, CV1, 3T3, and HeLa cell cultures served as controls. All were examined for SV40 antigens by indirect immunofluorescence staining. Rabbit antiserum was used to test for SV40 viral capsid antigen, and hamster anti-T serum for SV40 tumor antigen. Cell fusion studies were done with two meningiomas that had detectable T-antigen, and one that did not. The meningioma cells were incubated with 8-azaguanine, and fused with the SV40-permissive African green monkey kidney MA 134 cell line by means of inactivated Sendai virus. Heterokaryons were detected by labeling the meningioma cells with [^{3}H]thymidine. A positive fluorescence reaction characteristic for SV40 tumor antigen was found in two of the seven meningiomas. The percentage of positive nuclei varied with culture passage, one tumor had 90% in the first passage; and 40% in the fourth. One tumor tested in the fifth passage, presented a perinuclear fluorescence similar to the SV40 U-fluorescence in about 10% of the cells. None of the controls gave a positive reaction None of the cultures showed viral capsid antigen fluorescence. Three of the tumors were missing chromosome 22, and two of these were also missing four or five other chromosomes. Cell fusion resulted in 20.4 -24.0% of the nuclei being found in heterokaryons, with about 2.75 nuclei per heterokaryon. A low percentage (0.01-0.05%) of viral capsid antigen-positive nuclei occurred seven days after cell fusion. Cocultivation of tumor and MA 134 cells without fusion gave negative results for viral capsid antigen. It is concluded that there may be a correlation between meningioma and the presence of SV40-related papova virus.

1442 CHARACTERIZATION OF AN SV40-TRANSFORMED 3T3 CELL LINE EXPRESSING AN UNUSUAL PHENOTYPE. (Eng.) Thompson, J. E. (Dept. Biol., Univ. Waterloo, Waterloo, Ontario, Canada); Elligsen, J. D.; Frey, H. E. *J. Cell Sci.* 18(3):427-440; 1975.

A transformed variant derived as a clone from normal 3T3 cells infected with simian virus 40 (SV40) was found to possess a phenotype intermediate between that of normal cells and that characteristic of the transformed state, yet cells of the variant still tested positively for the SV40-specific nuclear T-antigen. The variant exercised growth control, although not as stringently as did normal cells. Its cell size more closely resembled that of normal cells than of transformed cells. The variant also exhibited levels of spontaneous agglutination that were in accordance with those characteristic of the normal cells from which it was derived, and were far higher than corresponding values for cells exhibiting the fully transformed phenotype. Plasma membranes of variant cells more closely resembled those of transformed cells than of normal cells as estimated by polyacrylamide gel electrophoresis. Perhaps the most distinguishing characteristic of the transformed variant was its complete immunity to agglutination by concanavalin A (Con A), even at concentrations as high as 500 μg/ml. Moreover, trypsinization did not render variant cells as agglutinable in the presence of Con A as untreated fully transformed cells. By contrast, the variant displayed a low tolerance to Con A toxicity, as monitored by ability to grow after treatment, and in this respect resembled transformed cells. Moreover, a survey of several normal cell lines revealed that even they do not consistently show resistance to Con A toxicity. These observations indicate that Con A-mediated agglutination and inability to grow after treatment with Con A are quite independent and do not have a cause and effect relationship.

1443 INHIBITION OF EMBRYONIC CELL AGGREGATION BY NEOPLASTIC CELLS. (Eng.) Maslow, D. E. (Dep. Exp. Pathol., Roswell Park Mem. Inst., Buffalo, N.Y.); Mayhew, E. *J. Natl. Cancer Inst.* 54(5):1097-1102; 1975.

The effects of "normal" and malignant cells on embryonic cell aggregation have been compared with the use of the gyratory shaker culture system. Simian virus 40 (SV40)-transformed BALB/3T3 cells or cells of an untransformed ("normal") 3T3 mouse line were added to cultures of neural retina cells from chicken embryos. Addition of the transformed cells caused a highly significant greater reduction in aggregate diameter than the addition of untransformed cells. The ratio of the number of single cells to the number of aggregates was also significantly higher for cultures containing the malignant cells than for cultures containing "normal" cells. Cultured Ehrlich-Lettré hyperdiploid ascites cells had the same effect on the neural retina cells as the SV40-transformed cells and this effect was shown to be dependent on the concentration of the ascites cells. However, media from the ascites cell cul-

tures or their cell sonicates had effects opposite those of the intact cells. This method is a sensitive assay for the investigation of the interaction of tumor with normal cells. Possible explanations for the inhibition of aggregation by tumor cells are discussed.

1444 REGULATION OF TUMOR ANTIGEN SYNTHESIS BY SIMIAN VIRUS 40 GENE *A*. (Eng.) Tegtmeyer, P. (Dept. Microbiology, State Univ. New York Stony Brook, Stony Brook, N.Y. 11794); Schwartz, M.; Collins, J. K.; Rundell, K. *J. Virol.* 16(1):168-178; 1975.

The effect of the simian virus 40 gene *A* function on proteins synthesized during productive and transforming infections is examined. Under restrictive conditions, temperature-sensitive *A* mutants induce the overproduction of a 100,000-dalton protein both in productively infected monkey cells and in transformed rabbit cells. Immunoprecipitation of the induced protein with antisera, prepared against simian virus 40-induced tumors in hamsters, was used to identify the induced protein as tumor antigen. The same protein could be precipitated from extracts of cells infected by wild-type virus but not from uninfected cells. Furthermore, the mutant-induced protein was more rapidly degraded *in vivo* and was less tightly bound to intranuclear components than the protein induced by wild-type virus. The presence of the same virus-induced protein in infected cells from different species and the altered behavior of that protein in mutant infection strongly suggest that the protein is virus coded. Because the protein is large enough to account for the entire coding capacity in the early region of the simian virus 40 genome, the 100,000-dalton protein may well be the primary product of the only early gene identified by complementation studies, the *A* gene. If the 100,000-dalton protein that is overproduced in mutant infection is the *A* protein and the only early protein, then functional wild-type *A* protein must regulate its own synthesis in both productive and transforming infections.

1445 ANALYSIS OF DEFECTIVE SV40 DNA BY AGAROSE GEL ELECTROPHORESIS. (Eng.) Sol, C. J. A. (Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, Mauritskade 57, Amsterdam-O, The Netherlands); Walig, C.; ter Schegget, J.; van der Noordaa, J. *J. Gen. Virol.* 28(3):285-297; 1975.

Agarose gel electrophoresis was used to distinguish a limited number of size classes of simian virus 40 (SV40) DNA among the heterogeneous population of defective DNA molecules within the virus. Two stocks of SV40 were generated by undiluted serial passaging in BSC-1 cells; Set A contained defective virus particles, and set B contained no detectable amount of defective particles. The DNA purified from these cells was analyzed by low-voltage agarose gel electrophoresis, and mixtures of the open circular forms of SV40 DNA, ϕX 174 (RF) marker DNA, and PM_2 marker DNA were analyzed by electron microscopy. Electrophoresis of the set A viral DNA revealed nine DNA bands, each containing a specific size class of DNA. As determined by electron microscopy measurements, the different size classes were all shorter than the complete SV40 genome. The different classes of defective SV40 DNA were stable with respect to size during serial undiluted passage. Electrophoresis of the set B DNA also revealed defective virus DNA. When the DNA of sets A and B were coelectrophoresed it was found that the defective DNA in the two sets had both different and similar size classes, varying in length from 96-73% of the unit length SV40 DNA. The results demonstrate the sensitivity of the low-voltage agarose gel electrophoresis technique and suggest that it could serve as a method for the purification of a great number of defective variants of SV40.

1446 CHANGES IN LACTATE DEHYDROGENASE ENZYME PATTERN IN CHINESE HAMSTER CELLS INFECTED AND TRANSFORMED WITH SIMIAN VIRUS 40. (Eng.) Caltrider, N. D. (Univ. of Colorado Medical Sch., Denver, Colo.); Lehman, J. M. *Cancer Res.* 35(8): 1944-1949; 1975.

The lactate dehydrogenase (LDH) isozyme patterns of consecutive passages of normal and simian virus 40 (SV40)-transformed Chinese hamster embryo cultures (whole embryo and embryo kidney) were monitored by electrophoresis and compared. At early passages the population displayed two LDH bands, M_4 and M_3H; however, at higher passages the cultures exhibited M_2H_2, M_3H, and M_4. When primary cultures of Chinese hamster embryo cells were infected with SV40, no change in the LDH pattern was observed; however, the total activity of LDH increased. Twenty-three of 25 transformed colonies isolated from SV40-infected primary cells by their ability to grow in methyl cellulose produced only M_4 or M_4-M_3H isozymes bands. Four of the SV40-transformed clones that produced only the M_4 isozyme were tested for LDH activity and found to have activities 2.5-3 times greater than the control cells. Kidney epithelial cells transformed with SV40 virus had a decrease in the H subunit production from 57-31%, compared with untransformed cells. This decrease led to an increase in the cathode-migrating isozymes. Therefore, a shift to the cathode-migrating isozyme was observed in SV40-transformed cells. The change in LDH isozyme patterns may be related to the acquisition of a more fetal LDH pattern in neoplastic cells.

1447 DIFFERENTIAL HEAT RESPONSE OF NORMAL AND TRANSFORMED HUMAN CELLS IN TISSUE CULTURE. (Eng.) Kase, K. (Stanford Univ. Sch. Med., Calif.); Hahn, G. M. *Nature* 255(5505):228-230; 1975.

The response of normal cells and similar malignant cells to hyperthermic treatment was compared. Embryonic lung fibroblasts WI-38 and simian virus 40 (SV40)-transformed WI-38-VA13/2RA were cultured; cells from the exponential phase were picked and grown to plateau-phase and then incubated at 43 C. The transformed cells exhibited a lower survival rate after hyperthermia than did normal cells under the same conditions. Population kinetics during and after exposure to 43 C were studied by measuring changes in the DNA distribution of exponentially

growing cells after heating. The accumulation of cells at 43 C in the S-phase, together with a depletion of cells in the G2-M, may indicate a heat-caused block from S to G2. The depletion of G1 also indicates a mitotic block during heating. Since transformed cells are more easily destroyed by hyperthermia and their cell kinetics change after heating, treatment by hyperthermia alone or in combination with other therapy may improve the therapeutic ratio.

1448 METHYLATED SIMIAN VIRUS 40-SPECIFIC RNA FROM NUCLEI AND CYTOPLASM OF INFECTED BSC-1 CELLS. (Eng.) Lavi, S. (Roche Inst. Molecular Biology, Nutley, N. J. 07110); Shatkin, A. J. *Proc. Natl. Acad. Sci. USA* 72(6):2012-2016; 1975.

Simian virus 40 (SV40)-specific RNA was examined for the presence of methylated nucleotides. African green-monkey kidney cells (BSC-1) were infected with SV40 and labeled with L-(*methyl*-^{3}H)methionine. Host cell and virus-specific poly(A)-containing RNAs, isolated from nuclei and cytoplasm of the infected cells, contained different methylated nucleotides. In the cytoplasmic SV40-specific RNA, about 75% of the radioactivity was in N^6-methyladenosine (N^6mA) after digestion with *Penicillium* nuclease and bacterial alkaline phosphatase. The remainder was in a negatively charged component with properties of 5'-terminal structures, i.e., digestion with nucleotide pyrophosphatase and bacterial alkaline phosphatase released 2'-*O*-methyladenosine (A^m), 2'-*O*-methylguanosine (G^m), and 7-methylguanosine (m^7G), consistent with a 5'-terminal structure of the type, m^7GpppN^m. The nuclear virus-specific RNA contained N^6mA, G^m, 2'-*O*-methyluridine, and a smaller proportion (10%) of nuclease-, phosphatase-resistant presumptive 5' termini that also yielded A^m, G^m, and m^7G upon further hydrolysis. The infected cell nuclear and cytoplasmic RNAs that did not hybridize to DNA of SV40 contained all four 2'-*O*-methylnucleosides. The results indicate that SV40-specific mRNA is methylated and that the pattern of methylation is different in nuclear and cytoplasmic virus-specific RNAs.

1449 PROPERTIES OF THE GENOME IN NORMAL AND SV40 TRANSFORMED WI38 HUMAN DIPLOID FIBROBLASTS. I. COMPOSITION AND METABOLISM OF NONHISTONE CHROMOSOMAL PROTEINS. (Eng.) Krause, M. O. (Dept. Biochemistry, Univ. Florida, Gainesville, Fla. 32610); Kleinsmith, L. J.; Stein, G. S. *Exp. Cell Res.* 92(1):164-174, 1975.

The composition and metabolism of nonhistone chromosomal proteins associated with the genome of normal and simian virus 40 (SV40)-transformed WI38 human diploid fibroblasts were examined. Variations in the relative protein content, specific activity and phosphorylation of several defined molecular weight classes of these chromosomal polypeptides were indicated. In addition, it was shown that blocking DNA replication with cytosine arabinoside did not inhibit the incorporation of ^{3}H-leucine into nonhistone chromosomal proteins of SV40-transformed cells. The possibility that the nonhistone chromosomal proteins of human diploid fibroblasts transformed by SV40 virus may be involved in the regulation of viral induced alterations in gene expression is discussed.

1450 A TRANSCRIPTIONAL MAP OF THE SV40 GENOME IN TRANSFORMED CELL LINES. (Eng.) Khoury, G. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.); Martin, M. A.; Lee, T. N. H.; Nathans, D. *Virology* 63(1):263-272; 1975.

An analysis of viral DNA transcription in several simian virus 40 (SV40)-transformed BSC-1 cell lines indicated the presence of both abundant and scarce classes of viral specific RNA. The topographical location of the SV40 DNA sequences which were complementary to transformed cell RNA was determined in hybridization experiments utilizing the separated strands of the 11 SV40 DNA fragments produced by digestion with the *Hemophilus influenzae* (R·*Hind*) restriction endonuclease. Extensive hybridization of the transformed cell RNA with the minus DNA strands of *Hin* fragments A, H, I, and B indicated that the abundant species of viral-specific RNA was similar, if not identical, to early SV40 RNA. The less abundant class of viral RNA was complementary to regions of the minus DNA strand contiguous with the early region, particularly those sequences located at the 3'-DNA end of this segment of the genome. Viral-specific RNA, complementary to the plus DNA strand and present in low concentrations in only a few transformed cell lines, could not be localized on the genome.

1451 ENHANCEMENT OF THE REPLICATION OF HUMAN ADENOVIRUS IN SIMIAN CELLS BY A SERIES OF TEMPERATURE-SENSITIVE MUTANTS OF SIMIAN VIRUS 40. (Eng.) Jerkofsky, M. (Milton S. Hershey Med. Cent. Pennsylvania State University, Hershey). *Virology* 65(2):579-582; 1975.

Representative members of each of the five complementation groups of simian virus 40 (SV40) temperature-sensitive mutants were tested for their ability to enhance the replication of human adenovirus type 7 in primary African green monkey kidney cells at 37 C and 41.5 C. Temperature-sensitive mutants of complementation groups A, B, C, and BC were found to enhance the replication of the adenovirus at nonpermissive temperatures, whereas members of group D could not. The available temperature-sensitive mutants of SV40 are apparently unable to delineate the cistron responsible for the enhancement of adenovirus replication in simian cells.

1452 STUDIES ON THE *IN VITRO* REASSEMBLY OF SV40 VIRUS-LIKE PARTICLES [abstract]. (Eng.) Christensen, M. L. (Northwestern Univ., Evanston, Illinois). *Diss. Abstr. Int. B.* 35(10): 5005; 1975.

1453 PATTERNS OF MACROMOLECULAR SYNTHESIS AND MITOSIS IN HUMAN DIPLOID CELLS, STRAIN WI-38: EFFECT OF SENESCENCE AND SV_{40} TRANSFORMATION [abstract]. (Eng.) Neff, N. L. T. (Stanford Univ., Palo Alto, California). *Diss. Abstr. Int. B* 35(10):4771; 1975.

1454 NICOTINAMIDE NUCLEOTIDE LEVELS IN NORMAL AND TRANSFORMED CELLS [abstract]. (Eng.) Jacobson, E. L. (North Texas State Univ., Denton); Jacobson, M. K. *Fed. Proc.* 34(3):589; 1975.

1455 REVERSION OF A TEMPERATURE SENSITIVE (ts) MUTANT OF SV40 VIRUS BY ULTRAVIOLET (UV) .IGHT [abstract]. (Eng.) Cleaver, J. E. (Lab. Radiobiol., Univ. California, San Francisco). *Radiat. Res.* 62(3):532; 1975.

456 TEMPORAL ORDER OF SYNTHESIS OF COMPONENT α DNA DURING MONKEY DNA SYNTHESIS INDUCED 3Y SV40 VIRUS [abstract]. (Eng.) Parker, R. J. Albert Einstein Coll. Med., Bronx, N.Y.); Tobia, ı. M.; Baum, S. G.; Schildkraut, C. I. *Fed. Proc.* 34(3):494; 1975.

457 BIOCHEMICAL EVIDENCE FOR A VIRAL INVOLVEMENT IN HUMAN BREAST CANCER [abstract]. Eng.) Colcher, D. M. (Columbia Univ., New York, .Y.). *Diss. Abstr. Int. B.* 35(10):4766-4767; 975.

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459 CONCERNING THE ACTION MECHANISM OF FREUND'S ADJUVANT IN VIRAL LEUKOMOGENESIS. Rus.) Bergolts, V. M. (Lab. Exper. Tumor Ther., . A. Gertsen Moscow Inst. Oncol., U.S.S.R.); Tolacheva, E. N.; Sukhin, G. M.; Sokolov, P. P.; ovalev, B. N. *Probl. Gematol. Pereliv. Krovi*)(2):42-48; 1975.

460 SIMPLE ASSAY OF MURINE LEUKEMIA VIRUSES USING RFL-CELLS. (Jpn.). Koga, M. Faculty Medicine, Kyushu Univ., Fukuoka, Japan). *ıkuoka Acta Med.* 66(2):83-91; 1975.

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62 THE PURIFICATION OF RNA TUMOR VIRUS-LIKE PARTICLES FROM HUMAN LEUKEMIC TISSUE ıbstract]. (Eng.) Baxt, W. G. (Dept. Med., Univ. ılifornia, San Diego). *Clin. Res.* 23(3):400A; 75.

63 CELL CYCLE DEPENDENCE OF 5-BROMODEOXYURIDINE EFFECTS ON RAT EMBRYO CELL DNA bstract]. (Eng.) Schwartz, S. A. (Univ. Chicago, 1.); Kirsten, W. H. *Fed. Proc.* 34(3):871; 1975.

1464 INDUCTION OF SARCOMAS IN MICE BY SV40 VIRUS [abstract]. (Eng.) Hargis, B. J. (Sidney Farber Cancer Cent., Boston, Mass.); Malkiel, S. *Fed. Proc.* 34(3):973; 1975.

1465 EFFECT OF INTERFERON ON GROWTH AND DIVISION CYCLE OF MURINE ERYTHROLEUKEMIC CELLS *IN VITRO* [abstract]. (Eng.) Rossi, G. B. (Inst. Superiore di Sanita, 00161 Rome, Italy); Matarese, G. P.; Grappelli, C. *Fed. Proc.* 34(3): 960; 1975.

1466 CONTINUOUS *IN VITRO* REPLICATION OF LEUKEMOGENIC BUT NONIMMUNOSUPPRESSIVE FRIEND LEUKEMIA VIRUS COMPLEX IN MOUSE EMBRYO FIBROBLASTS [abstract]. (Eng.) Eckner, R. J. (Boston Univ. Sch. Med., Mass.) *Fed. Proc.* 34(3):973; 1975.

1467 FURTHER COMPARISONS BETWEEN TISSUE CULTURE AND MOUSE PLASMA DERIVED FRIEND VIRUS [abstract]. (Eng.) Evenson, D. P. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Pla, D. M.; de Harven, E. *Fed. Proc.* 34(3):974; 1975.

1468 PHYSICAL CHARACTERISTICS OF HERPESVIRIONS: LOW-TEMPERATURE AND OSMOTIC-SHOCK STUDIES. (Eng.) Barnhart, E. R. (Dept. Microbiology, Emory Univ., Atlanta, Ga. 30322); Ash, R. J. *Virology* 66(2):563-567; 1975.

1469 HERPES SIMPLEX VIRUS TYPE 2 INFECTION OF SYNCHRONIZED KB CELLS [abstract]. (Eng.) Kelman, A. D. (Boston Univ. Sch. Med., Mass.); Kibrick, S. *Fed. Proc.* 34(3):870; 1975.

1470 HOST-VIRUS RELATIONSHIPS IN VIRAL LEUKEMIA: GENETIC CONTROL OF THE DISEASE. (Fre.) Levy, J.-P. (Laboratoire d'Immunologie des Tumeurs, Service d'Hematologie, Hopital Cochin, 27, rue du Faubourg Saint-Jacques, F. 75014 Paris, France). *Bull. Cancer (Paris)* 62(2):213-220; 1975.

1471 THE NEONATAL STATE IS NOT OBLIGATORY FOR INFECTION OF MICE WITH MURINE LEUKEMIA VIRUS [abstract]. (Eng.) Levy, R. L. (Scripps Clin. Res. Found., La Jolla, Calif.) Lerner, R. A.; Dixon, F. J. *Fed. Proc.* 34(3):974; 1975.

1472 SPLENOCYTE PLAQUE ASSAY FOR THE DETECTION OF MURINE LEUKEMIA VIRUS. (Eng.) Melief, C. J. M. (New England Medical Cent. Hosp., Boston, Mass. 02111); Datta, S. K.; Louie, S.; Johnson, S.; Melief, M.; Schwartz, R. S. *Proc. Soc. Exp. Biol. Med.* 149(4):1015-1018; 1975.

1473 TRANSPLANTATION RESISTANCE TO ADENOVIRUS-INDUCED HEPATOCELLULAR CARCINOMAS. (Eng.) McCormick, K. J. (Baylor Coll. of Medicine, Houston, Tex. 77025); McCormick, N. K.; Trentin, J. J. *Proc. Soc. Exp. Biol. Med.* 149(4):1044-1047; 1975.

1474 NEOPLASTIC RESPONSE OF THE AVIAN LIVER TO HOST INFECTION WITH STRAIN MC29 LEUKOSIS VIRUS. (Eng.) Beard, J. W. (Life Sciences Res. Lab., St. Petersburg, Fla. 33710); Hillman, E. A.; Beard, D.; Lapis, K.; Heine, U. *Cancer Res.* 35(7): 1603-1627; 1975.

1475 THE INDUCTION OF INTRACRANIAL NEOPLASMS BY THE INOCULATION OF AVIAN SARCOMA VIRUS IN PERINATAL AND ADULT RATS. (Eng.) Copeland, D. D. (Natl. Inst. of Environmental Health Sciences, Research Triangle Park, N.C. 27709); Vogel, F. S.; Bigner, D. D. *J. Neuropathol. Exp. Neurol.* 34(4): 340-358; 1975.

1476 C-TYPE VIRUS PARTICLES IN A CELL LINE FROM A LYMPHOSARCOMA OF A NUDE MOUSE. (Eng.) Tralka, T. S. (Natl. Cancer Inst., Bethesda, Md.); Rabson, A. S.; Hansen, C. T. *J. Natl. Cancer Inst.* 55(1):197-198; 1975.

1477 NATURALLY OCCURRING CYTOTOXIC TUMOUR REACTIVE ANTIBODIES DIRECTED AGAINST TYPE C VIRAL ENVELOPE ANTIGENS. (Eng.) Martin, S. E. (Natl. Lung and Heart Inst., Natl. Inst. Health, Bethesda, Md. 20014); Martin, W. J. *Nature* 256(5517):498-499; 1975.

1478 ANTIBODY IN CATS TO MAMMALIAN RNA TUMOR VIRUS INTERSPECIES ANTIGENS. (Eng.) Olsen, R. G. (Dept. Veterinary Pathobiology, Ohio State Univ., Columbus, Ohio 43210); Mathes, L. E.; Yohn, D. S. *Cancer Res.* 35(9):2580-2585; 1975.

1479 VIRUS CELL CHANGE RELATIONSHIPS IN VIRAL LEUKEMOGENESIS. (Ger.) Fey, F. (Institut fur Medizin u. Biologie, Lindenberger Weg 70, Berlin-Buch, East Germany); Niezabitowski, A.; Graffi*, A. *Haematologia (Budap.)* 8(1-4):29-34; 1974.

See also:

* (Rev): 1209, 1210, 1211, 1212, 1213, 1227, 1228, 1229, 1230, 1239
* (Phys): 1455, 1468
* (Immun): 1481, 1491, 1492, 1493, 1496, 1497, 1503, 1507, 1511, 1512, 1537, 1538, 1539, 1540, 1543, 1561, 1563, 1572, 1591, 1592, 1598
* (Path): 1629
* (Epid-Biom): 1703

1480 USE OF THE DEFINED ANTIGEN SUBSTRATE SPHERES SYSTEM AS A MODEL FOR ANALYSING POSSIBLE MECHANISMS OF INHIBITION-BLOCKADE OF ANTI-TUMOUR LYMPHOCYTOTOXICITY. (Eng.) Matthews, N. (Dep. Pathol. Immunol., Monash Univ., Melbourne, Australia); de Kretser, T.; Nairn, R. C. *Immunology* 28(6):1081-1087; 1975.

The defined antigen substrate spheres system was employed to analyze reactions between solid state antibody or antigen and soluble immune complexes. Sepharose beads covalently coupled with ovalbumin were used to represent tumor cells, and beads coupled with antibody against ovalbumin were used to represent anti-tumor lymphocytes; the ovalbumin and corresponding antibody simulated tumor-derived antigen and antibody to tumor, respectively. Binding of soluble complexes to the beads was measured by fluorimetry and/or radiometry of fluorescein or ^{125}I-labeled ovalbumin or antibody. Antigen-antibody complexes in antibody excess bound less effectively to the antibody beads than antigen alone, but complexes in slight or moderate antigen excess bound more effectively. Complexes in antibody excess were most effective in binding to antigen beads binding increased with the amount of antibody in the complex before leveling and then decreased in extreme antibody excess. The model demonstration of augmentation by antibody of antigen binding to solid state antibody might by analogy reflect a mechanism of inhibition of lymphocyte cytotoxicity. If these results are applicable to tumor immunity, then complexes of tumor-derived antigen with anti-tumor antibody would bind to tumor cell surfaces and protect against attack by cytotoxic lymphocytes

481 THE SPECIFICITY OF CELLULAR IMMUNE REACTIONS TO THREE DNA VIRUS INDUCED TUMOUR S MEASURED BY THE MACROPHAGE MIGRATION INHIBITION EST. (Eng.) Rees, R. C. (Univ. Sheffield Med. Sc ngland); Potter, C. W.; Shelton, J. *Eur. J. Cance* 1(2):79-86; 1975.

yrian hamsters were immunized with x-irradiated tu or cells (three wkly injections) of adenovirus 12, ELO- or SV40-induced tumors to test for cross-reac ng antigens in extracts of the tumor cells. Peritoneal exudate cells were collected from immunized nimals five days following the i.p. injection of .0 ml of paraffin oil. These cells were tested fc ellular reactivity against homologous and heterlogous tumor cell extracts using the macrophage mi ration inhibition (MMI) test. Immunization with umor cells provided significant protection against omologous viral challenge for all three types of ells. Macrophage migration was inhibited by 10% v/v) homologous tumor extract in cells obtained rom SV40 or CELO tumor cell-immunized hamsters. acrophages from adenovirus 12 tumor cell-immunized amsters were inhibited by a 10% SV40 tumor extract nd by 2% and 10% homologous tumor extracts. The esults indicate that the MMI test may show specificity similar to that of transplantation immunity ests, and that lymphoid cells obtained from adenoirus 12 tumor cell-immunized hamsters are sensitive antigen(s) that is present in SV40 tumor cell extract. The authors suggest that these antigens may be similar to embryonic antigens which are common in different tumors.

1482 SUBPOPULATIONS OF MULTIPAROUS RAT LYMPH-NODE CELLS CYTOTOXIC FOR RAT TUMOUR CELLS AND CAPABLE OF SUPPRESSING CYTOTOXICITY *IN VITRO*. (Eng.) Rees, R. C. (Cancer Res. Campaign Lab., Univ. Nottingham, University Park, England); Bray, J.; Robins, R. A.; Baldwin, R. W. *Int. J. Cancer* 15(5): 762-772; 1975.

Lymph node cells (LNC) from multiparous, pregnant syngeneic Wistar rats were separated on columns prepared from nylon wool, and tested for cytotoxicity against target tumor cells. Reactivity of LNC toward hepatoma D23 and mammary carcinoma AAF57 was demonstrated in cell populations retained on the nylon wool, and not those eluted from the column. Although only 25% of the samples of unfractionated LNC were cytotoxic for tumor cells, retained cell fractions were cytotoxic in 11 out of 12 tests. Similarly retained LNC were also cytotoxic for 15-day-old embryo cells but not for normal adult rat fibroblasts. Using multiparous rat serum, the reactivity of the retained LNC population could be abrogated in eight out of 11 tests. The LNC population recovered from the nylon wool constituted 28-35% of the original LNC preparation, and consisted of 60-70% Ig-bearing cells together with a subpopulation of cells responding to soluble phytohemagglutinin (PHA). Approximately 17-20% of the original cell population was recovered from cells retained on the column, and consisted of an enriched Ig-bearing cell population (65-80% Ig-bearing cells) and LNC responsive to PHA. Carbonyl iron treatment of multiparous rat LNC was found to remove detectable cytotoxicity from multiparous rat LNC preparations. The cytotoxicity of multiparous rat LNC retained on nylon wool was also abolished following incubation with carbonyl iron. Carbonyl iron treatment removed not only phagocytic cells from LNC preparations, but also a proportion of other cell populations including Ig-bearing lymphocytes. In addition to detecting a cytotoxic LNC population reactive towards tumor-associated embryonic antigens (retained fraction from column), a subpopulation of multiparous rat LNC was demonstrated in cell fractions eluted from the nylon wool which was shown to suppress the cytotoxicity of the retained multiparous LNC population. The results show that multiparous rat LNC include a subpopulation capable of abrogating lymphocytotoxic reactions against the embryonic antigen component.

1483 POTENTIATION OF THE TUMOR-SPECIFIC IMMUNE RESPONSE BY *CORYNEBACTERIUM PARVUM*. (Eng.) Scott, M. T. (Trudeau Inst., Inc., Saranac Lake, N.Y.). *J. Natl. Cancer Inst.* 55(1):65-72; 1975.

The strong, specific, cell-mediated antitumor immunity that results from the sc injection into adult male C57BL/6 x DBA/2)F_1 mice of 175 μg of *Corynebacterium parvum* (CP) mixed with 10^7 irradiated mastocytoma P815 (MA) cells is described. Injection of irradiated MA alone was without effect. Maximum immunity was achieved with small doses of CP,

and the injection sites of CP and irradiated MA did not need to be coincident but required common lymphoid drainage for immunity to result. The process was largely T-cell-dependent but was not dependent on the development of a specific immune response to CP; it was also demonstrable and effective in a therapy situation. No such immunity was evident after systemic administration of CP and irradiated MA. Simultaneous systemic injection of CP and irradiated MA did, however, nonspecifically reduce the enhancing effect of systemic injection of irradiated MA alone. The local antitumor effects of an intratumor injection of CP may be due to either a nonspecific effect arising from a specific immune response to CP or to the specific immune destruction of tumor resulting from the interaction of CP with tumor antigen. It is suggested that further manipulation of the variables involved (e.g., multiple injections, timing and alternate means of tumor inactivation) may increase the effectiveness of mixed vaccines.

1484 HISTOCOMPATIBILITY ANTIGENS ACTING AS HELPER DETERMINANTS FOR TUMOR-ASSOCIATED ANTIGENS OF MURINE LYMPHOSARCOMA. (Eng.) Colnaghi, M. I. (Division of Experimental Oncology A, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, I-20133 Milano, Italy). *Eur. J. Immunol.* 5(4):241-245; 1975.

The mechanism of effectiveness of allogeneic *versus* syngeneic immunization in mounting a humoral specific immune response against tumor-associated antigens of murine urethan-induced thymic lymphosarcomas was studied. C57BL mice were injected five times with tumor or normal cells (half sc and half ip) at 1- to 2-wk intervals, the first inoculum being 5 x 10^6 cells and the following doses increasing 2-fold. Mice immunized with untreated or blocked C3Hf lymphoma cells yielded sera with complement-dependent cytotoxicity for C57BL lymphosarcomas, whereas they did not respond to repeated injections of blocked syngeneic lymphoma cells, indicating a helper activity of alien alloantigens in the reflex of tumor antigens. Thymectomized, lethally-irradiated and T, but not B-reconstituted mice, produced active sera. The anti-tumor and anti-histocompatibility activities of the serum ran parallel. The tumor-associated and the alien histocompatibility determinants had to be present on the same cell membrane. In addition, active sera were obtained when immunizing syngeneic lymphoma cell inocula were followed by allogeneic normal immunocompetent cells. It is suggested that either a helper mechanism with B and T cells cooperating in recognizing haptenic tumor-associated and carrier histocompatibility antigens or a graft-*versus*-host reaction determining an abnormal induction of the immune system is needed in order to detect the weak tumor-associated antigens of urethan-induced lymphomas.

1485 METASTASIS FORMATION OF YOSHIDA SARCOMA HETEROTRANSPLANTED IN ADULT GOLDEN HAMSTERS TREATED WITH ANTI-HAMSTER THYMOCYTE SERUM. (Eng.) Sakakibara, K. (Fac. Med., Univ. Tokyo, Japan); Oota, K. *Cancer Res.* 35(3):548-553; 1975.

The effect of host-conditioning methods on the biological behavior of xenografted neoplasms in the adult noninbred Syrian golden hamster was studied. Yoshida sarcoma cells, used as the xenograft, were inoculated into the submucosal layer of the cheek pouches (0.1 ml inoculum containing 10^6 cells). The animals were divided at random into three groups of 24. The experimental group received antithymocyte serum (ATS), 0.5 ml/animal; one control group received hydrocortisone acetate, 2.5 mg/animal; and the other control received nothing. Each group was divided into eight small groups and received s.c. injections of the conditioning substances twice weekly. Three hamsters from each group were sacrificed on days 1, 3, 5, 7, 14, 21, 28, and 35 and the cheek pouches were examined. In the ATS-treated group, tumors of cheek pouches grew progressively until day 21 with no sign of regression. Little host reaction was noticed histologically around the graft. Distant metastases were revealed at autopsy in nine of 12 hamsters from day 14 on and all died by day 23. Six of the nine animals with metastases died of tumor. The back-transplantation test revealed metastatic tumor cells consistent with Yoshida sarcoma cells. In the hydrocortisone-treated group and the untreated group, the tumor reached maximum size by day 21 and day 5, resp.; regression then followed. No metastases were detected in either control group. Treatment of hamsters with ATS resulted in an unexpectedly high incidence of distant metastases of Yoshida sarcoma accompanied by progressive tumor growth at the inoculum site.

1486 TRANSPLANTABILITY OF L1210 CELL IN BALB/C X DBA/$2F_1$ MICE ASSOCIATED WITH CELL AGGLUTINABILITY BY CONCANAVALIN A. (Eng.) Kataoka, T. (Jap. Found. Cancer Res., Tokyo, Japan); Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* 35(3):531-534; 1975.

The effect of different concentrations of glutaraldehyde at ice-cold temperatures of L1210 mouse leukemia cells was studied. The effect on cell surface was measured by trypan blue exclusion and cell agglutinability was measured by concanavalin A. The association of transplantability of glutaraldehyde-treated L1210 cell to cell agglutinability measured by concanavalin A was also examined. L1210 cells plus 0.013% glutaraldehyde caused a loss in transplantability in BALB/c X DBA/$2F_1$ mice and, concomitantly, a loss in agglutinability. The cells remaine able to exclude trypan blue up to concentrations of 0.2% glutaraldehyde; their surfaces were similar to intact cells in susceptibility to mechanical disruption and in absorption of cytotoxic activity of rabbi anti-L1210 cell serum. Transplantability was closel associated with cell agglutinability but not with tr pan blue exclusion. Thus, glutaraldehyde modified L1210 cells in two ways: at low concentrations it r duced agglutinability and at high concentrations it resulted in the loss of susceptibility to immune lysis. An experimental condition is suggested in whic immunization could safely induce resistance against L1210 leukemia.

1487 CORRELATION BETWEEN RESISTANCE TO ACTINOMYCIN D, KARYOLOGY, AGGLUTINATION BY CONCANAVALIN AND TUMORIGENICITY IN CHINESE HAMSTER HY-

BRID CELLS. (Eng.) Imbert, I. (Unite de Recherche de l'I.N.S.E.R.M. (U.199) 27, Bd LeI Roure, Marseilles, France); Barra, Y.; Berebbi, M. *J. Cell Sci.* 18(1):67-77; 1975.

Subclones isolated from a Chinese hamster hybrid line (HyC) were studied to determine possible relationships between various cell properties and tumorigenicity. The hybrid was derived from somatic hybridization of strain DC-$_3$F and strain DC-$_3$F/ADX/Aza. Strain DC-$_3$F had a karyotype of 21 chromosomes and regularly produced tumors in cortisone-treated Syrian hamsters; DC-$_3$F/ADX/Aza was derived from strain DC-$_3$F but was resistant to actinomycin D, had a modal karyotype of 22-23 chromosomes with a subtelocentric marker chromosome, M_1, and lacked tumor-inducing properties in Syrian hamsters. Shortly after fusion of the parental strains of cells, a subtelocentric chromosome characteristic of HyC, designated M_2, appeared. Clones were isolated from HyC by selective growth in soft agar. One clone, having a mode of 37 chromosomes and possessing the M_2 in about 50% of the mitoses, was selected for particular study. Five subclones were isolated from this clone on glass, and the different subclones were compared with respect to resistance to actinomycin D, karyology, transplantability, and agglutination by concanavalin A. The strains were classified on the basis of increasing resistance to actinomycin D by statistical analysis. There was an inverse correlation between actinomycin D resistance and tumorigenicity and a positive correlation between this resistance and the presence of the marker M_1. It is concluded from this result that the M_1 chromosome carries information coding for resistance to actinomycin D. No correlation could be shown between the surface modifications, detected by agglutinability by concanavalin A, and resistance to actinomycin D.

488 CELL-MEDIATED IMMUNITY TO MELANOMA-ASSOCIATED ANTIGENS IN PATIENTS WITH OCULAR ALIGNANT MELANOMA. (Eng.) Char, D. H. (Natl. Caner Inst., Bethesda, Md.); Jerome, L.; McCoy, J. L.; erberman, R. B. *Am. J. Ophthalmol.* 79(5):812-816; 975.

leven patients with choroidal melanomas, 21 normal ubjects, and 10 patients with nonocular malignanies were tested for cell-mediated immunity to meanoma-associated antigens by an *in vitro* WBC miration inhibition assay that involved incubating ell suspensions of heparinized blood with soluble otassium chloride extracts. These extracts were repared from sterile melanoma tissue obtained at urgery from three patients with locally metastatic stemic malignant melanomas. Five of seven patients ith choroidal melanomas, who received two melanoma tracts, had significant WBC migration inhibition as mpared with none of 17 normal subjects and none of ven with other malignancies. Four of five melanoma tients receiving a third melanoma extract had sig-ficant WBC migration inhibition, as did three of ur normal controls; this indicates that the exact showed nonspecific inhibition. The data are agreement with the concept that patients with oroidal melanomas have cell-mediated immunity to mmon melanoma-associated antigens.

1489 IMMUNOLOGIC ALTERATIONS IN PATIENTS WITH PROSTATIC CARCINOMA. (Eng.) Brosman, S. (Harbor Gen. Hosp., Torrance, Calif.); Hausman, M.; Shacks, S. *J. Urol.* 113(6):841-845; 1975.

The state of cell-mediated immunity was evaluated in 41 patients with either local or metastatic prostatic carcinoma by determining their ability to demonstrate delayed cutaneous hypersensitivity with dinitrochlorobenzene and recall antigens, and to mount an inflammatory response to croton oil. Changes in monocyte chemotactic response were also measured. Monocyte chemotactic response was significantly depressed in the entire group of prostatic cancer patients as compared to noncancer controls. Patients with metastatic carcinoma had a greater defect than those with localized disease. After radical prostatectomy a group of patients with localized disease tended to show improvement in monocyte chemotactic response. The ability of patients to develop a delayed cutaneous hypersensitivity response was impaired and correlated with the clinical stage of the disease. Croton oil also impaired the ability of these cancer patients to mount an inflammatory response and showed a close correlation between clinical stage and course of disease. Changes in response to croton oil tended to precede alterations in dinitrochlorobenzene response. This study shows evidence of depressed immunocompetence in patients with prostatic carcinoma, which seems to relate to disease stage and progression.

1490 CELL-MEDIATED IMMUNITY *IN VIVO* AND *IN VITRO* BY CHICKENS WITH BCG OR MAREK'S DISEASE INFECTION AND THE EFFECTS OF BURSECTOMY AND THYMECTOMY ON THE EXPRESSION OF CELL-MEDIATED IMMUNITY. (Eng.) Fauser, I. S. (Michigan State Univ., East Lansing). *Diss. Abstr. Int. B* 35(9):4513-4514; 1975.

Cell-mediated immunity *in vivo* and *in vitro* was studied in intact, bursectomized, or thymectomized chickens. The agents used to immunize the chickens were (BCG) and Marek's disease virus. The *in vivo* test used was delayed skin reaction to old tuberculin (OT). The *in vitro* test used was detection of migration inhibition factor (MIF) in peripheral lymphocytes of immunized chickens, using tuberculoprotein (B-24) and Marek's disease antigen (A-antigen) as testing reagents. Precipitating antibody was also tested by the Ouchterlony method. Immunized intact chickens had detectable antibody, MIF, and delayed skin reactions. All thymectomized chickens had antibody, 3 of 7 had delayed skin reactions, and only 1 of 7 had detectable MIF. Only the bursectomized chickens had true delayed skin reactions to OT. *In vivo* graft-*versus*-host (GVH) reactions by blood WBC were greater for WBC from thymectomized donors than for WBC from intact or bursectomized donors. The results showed that detection of MIF in avian blood WBC correlated with delayed skin reactions and supported evidence for the thymic role in the development of delayed sensitivity and of MIF production. However, the results also indicate that the GVH reactivity by blood WBC may not be by the same population of thymic dependent cells as those capable of recruitment for MIF production.

1491 *IN VITRO* DEPRESSION OF CELLULAR IMMUNITY BY FRIEND VIRUS LEUKEMIC SPLEEN CELLS. (Eng.) Toy, S. T. (Dep. Microbiol., Thomas Jefferson Univ., Philadelphia, Pa.); Wheelock, E. F. *Cell. Immunol.* 17(1):57-73; 1975.

The effect of statolon in combination with the leukemosuppressive augmenting agent chlorite-oxidized oxyamylose (COAM) on restoration of spleen cell responsiveness to Concanavalin A (Con A) in mice (inbred female DBA/2 or C57B1) infected with Friend virus (FV) was studied. Mice were divided into two groups, one of which was infected i.p. with FV. Two days later, half of each group received an i.v. inoculation of COAM (0.5 mg/0.2 ml phosphate buffered saline); one day later they received an i.v. inoculation of Statolon (5 mg). On various days thereafter, spleens were removed from mice in each of the four groups and the cells were placed in culture with Con A and incubated. Murine spleen lymphocytes showed a marked depression in their response to Con A 14 days after FV infection. This depression was related to the appearance of FV antigen-containing cells in the spleen. Statolon treatment suppressed FV disease and restored lymphocyte response to Con A. The addition of as few as one leukemic spleen cell to every five normal spleen cells inhibited the Con A response of the latter cells. Normal spleen lymphocytes were not inhibited by the addition of FV or leukemic spleen cell culture fluid. Leukemic cells had an increased avidity for Con A and preferentially removed it from the medium. Increased medium concentrations of Con A did not overcome the depressive effects of the leukemic spleen cells on normal cells, indicating that the depressed normal cellular response to Con A was produced by the leukemic cells. The data suggest that this depressive effect is mediated by membrane-membrane contact between the two cell types. The demonstration that leukemic cells can depress cell-mediated immunity of normal spleen cells *in vitro* permits a study of immunodepression mechanisms by FV and restoration by Statolon under conditions in which cell populations and interactions can be controlled.

1492 DEPRESSION OF HUMORAL IMMUNITY TO SHEEP ERYTHROCYTES *IN VITRO* BY FRIEND VIRUS LEUKEMIC SPLEEN CELLS: INDUCTION OF RESISTANCE BY STATOLON. (Eng.) Weislow, O. S. (Dep. Microbiol., Thomas Jefferson Univ., Philadelphia, Pa.); Wheelock, E. F. *J. Immunol.* 114(1):211-215; 1975.

Spleen cells obtained from female DBA/2 mice infected three weeks previously with 1,000-2,000 LD50 of Friend virus (FV) were studied as a new *in vitro* system to examine the induction of immunosuppression by FV and the restoration of immunocompetence by statolon. Spleen cells (1×10^7) and 1×10^6 SRBC were suspended in 1.0 ml of culture medium and incubated for five days at 37 C in 5% CO_2. Cultures were examined for antibody plaque-forming cells (PFC) by localized hemolysis in gel. Mice were pretreated with statolon (5 mg iv). FV-immune serum was obtained from mice in remission from FV-induced leukemia. FV-leukemic cells (5×10^5) inhibited the *in vitro* response of normal spleen cells to SRBC (23 PFC/10^6 cells *versus* 460 PFC/10^6 cells in controls). Depression of the immune response by FV-leukemic cells occurred when cells were added between one hour before, and 12 hr after SRBC addition. FV-immune serum inhibited the immunodepressive effects of FV-leukemic cells (707 PFC/10^6 cells with FV-immune serum *versus* 49 PFC/10^6 cells with normal serum added). Statolon-treated normal spleen cells resisted immunodepression by FV-leukemic cells (85% of PFC of controls *versus* 36% without statolon pretreatment). FV-leukemic cells thus depress the primary immune response of normal spleen cells to SRBC *in vitro*. This immunodepression is blocked by FV-immune serum *in vitro* and by pretreatment with statolon prior to cell cultivation. Preparation of cell-free FV is necessary for further investigation of the immunosuppressive factors.

1493 IMMUNOSUPPRESSION *IN VITRO* INDUCED BY LEUKEMIA VIRUS-INFECTED SPLENOCYTES. (Eng.) Kamo, I. (Albert Einstein Medical Center, Philadelphia, Pa. 19141); Kateley, J. R.; Kaplan, G.; Friedman, H. *Proc. Soc. Exp. Biol. Med.* 148(2):383-386; 1975.

Since previous work had shown that addition of cell-free Friend leukemia virus (FLV) to normal spleen cell suspensions *in vitro* failed to depress the induction of plaque-forming cells by sheep RBC, it appeared that the virus *per se* did not influence antibody formation in the *in vitro* model system. The present study was carried out to test the effect of addition of spleen cells from FLV-infected mice (instead of addition of cell-free FLV) on antibody formation induced by sheep RBC in normal spleen cells. The mice used were strain BALB/c and *in vitro* immunization was performed in Marbrook tissue culture vessels containing 5×10^6 normal BALB/c splenocytes and 2×10^6 washed sheep RBC. Assays for antibody production were performed on spleen cells harvested from the Marbrook chambers, using the standard hemolytic plaque technique in agar gel. For inhibition experiments, infected splenocytes from BALB/c mice 7-10 days after FLV infection were added to individual culture chambers at the time of culture initiation. It was found that relatively small numbers of splenocytes from infected mice, even when present at a ratio of 1/500 normal spleen cells, significantly suppressed the *in vitro* immune response to sheep RBC. Heating the cells from infected mice for 30 min at 56 C abolished their immunosuppressive activity, indicating that living cells were necessary for the *in vitro* immunosuppression. However, the leukemic splenocytes did not have to be in direct contact with the normal spleen cells to suppress their responsiveness to sheep RBC, since separation of the two spleen cell population by a nucleopore filter of 0.45 μm diameter pore size still resulted in significant immunosuppression. Thus, it seemed likely that a factor from infected spleen cells inhibited the antibody response. Presumably this subcellular material was virus, since specific anti-FLV serum neutralized the inhibitory effects of the leukemic splenocytes. It was suggested that the lack of suppression obtained when cell-free FLV was tested for inhibitory action was due to noninfectious virus competing for possible virus-specific receptors on

-he normal immunocytes, or that an FLV-associated pro-luct being released from infected cells was responsi-ıle for the suppression obtained when viable leuke-ıic. splenocytes were used.

1494 CORRELATION OF "SNEAKING THROUGH" OF TUMOR CELLS WITH SPECIFIC IMMUNOLOGICAL IM-PAIRMENT OF THE HOST. (Eng.) Mengersen, R. (Hein-rich-Pette-Institut, D-2 Hamburg 20, Martinistra e 52, Fed. Rep. Germany); Schick, R.; Kolsch, E.* Eur. *J. Immunol.* 5(8):532-537; 1975.

ι study was carried out to determine the mechanism esponsible for the preferential take of tumors af-ter inoculation of experimental animals with low numbers of transplantable tumor cells. When BALB/c ıice were injected ip three times at weekly inter-vals with 10^2 to 10^3 X-irradiated (3000 rad) BM3 ıastocytoma cells and were challenged with 10^5 li-ving BM3 cells five days later, the animals showed he highest tumor incidence. Animals pretreated rith 10^4 to 10^7 irradiated cells showed apparent omplete immunity to challenge. The findings were onfirmed by results of experiments in which the hallenge dose was 3 x 10^4 living cells and also by esults of experiments in which mean death rate was etermined instead of tumor incidence. The speci-icity of the facilitation of tumor production was emonstrated by results of control experiments in hich the incidence of tumor production in animals hallenged with EL-4 lymphoma cells was found to be naffected by pretreatment of the animals with 3 x 0^4 living BM3 cells. This apparent lack of general mmunosuppression was substantiated by the finding hat there was no difference in the *in vitro* T cell-ediated cytotoxicity against the EL-4 lymphoma in roups of animals pretreated with saline or with 5 x 0^1 living BM3 cells. Additional tests demonstrated hat the induction of a primary as well as an anam-estic response was suppressed in animals previously njected with 10^2 living tumor cells and that the eterminant-specific suppression could be transferred y spleen cells, indicating an active maintenance of nresponsiveness. It was concluded that "sneaking hrough" of tumor cells is the result of specific immunological impairment of the host's immune system y subimmunogenic small-size inocula of tumor cells.

495 IMMUNODEPRESSION BY ROWSON-PARR VIRUS IN MICE: EFFECT OF ROWSON-PARR VIRUS AND RIEND LEUKEMIA COMPLEX INFECTIONS ON BACKGROUND NTIBODY-FORMING CELLS TO VARIOUS ERYTHROCYTES. Eng.) Bendinelli, M. (Inst. Microbiol., Univ. Pisa, aly); Toniolo, A.; Campa, M. *Infect. Immun.* 11(5): 024-1030; 1975.

ıe numbers of background antibody-forming cells BPFC) toward RBC of various species present in the mphoid organs of unimmunized susceptible BALB/c ıd resistant C57BL/6 mice were investigated at vari-ıs times after infection with Friend leukemia com-ex (FLC) or Rowson-Parr virus (RPV). Infection s performed by retro-orbital injection of 0.1 ml rus preparation. The cells were tested against eep, chicken, horse, donkey, rabbit, and human roup O) RBC. Both virus preparations induced an increase of BPFC numbers in both animal strains, but the rate and magnitude of the enhancements produced by RPV were much lower. The degree of potentiation varied with the specificity of the BPFC populations and was more pronounced in the spleen than in the lymph nodes and in BALB/c than in C57BL/6 mice. In the late stage of FLC infection, the numbers of splenic BPFC to some RBC underwent a dramatic fall, which was not observed in RPV-infected mice. BPFC present in BALB/c splenocytes cultured in diffusion chambers implanted in the peritoneal cavity of isogeneic normal mice were not affected by viral infection of the chambers. The results demonstrate that despite similarities between the effects of RPV and FLC infections on antibody response, other immunological parameters may be differently affected by the two virus preparations.

1496 SELECTIVE IMMUNOSUPPRESSIVE ACTIVITY OF STEROIDS IN MICE INOCULATED WITH THE MOLONEY SARCOMA VIRUS. (Eng.) Markham, R. B. (Univ. Texas Med. Branch, Galveston); White, A.; Goldstein, A. L. *Proc. Soc. Exp. Biol. Med.* 148(1):190-193; 1975.

The immunosuppressive activity of steroids was examined in mice inoculated with Moloney sarcoma virus. Eighty 5-wk-old female CBA/Wh mice were injected ip with 6.25 mg 6-chloro-17α-hydroxy-pregna-1,4,6-triene-3,20-dione (CHP), progesterone, cortisol, or 0.25 ml of vehicle. On the following day, the animals were injected im with 0.1 ml MSV diluted 1:10 with phosphate-buffered saline (pH 7.2). At three months after virus inoculation, 40% of the control mice, 35% of the CHP-treated mice, and 75% of the cortisol- and progesterone-treated mice had died. CHP was devoid of significant immunosuppressive activity in this model. CHP may be useful in modulating specific aspects of cellular immunity without altering others. This model represents a new, simple experimental method for assessing immunosuppressive effects of agents on viral-induced tumor growth.

1497 IMMUNIZATION WITH A LIPID-CONJUGATED MEMBRANE ANTIGEN TO SUPPRESS GROWTH OF A FIBROSARCOMA INDUCED BY SIMIAN VIRUS 40. (Eng.) Hunter, R. L. (Dept. of Pathology, Univ. of Chicago, Chicago, Ill. 60637); Strickland, F. *J. Natl. Cancer Inst.* 54(5):1157-1163; 1975.

Lipid conjugation procedures were used for chemical modification of a tumor-cell membrane preparation in order to stimulate a more effective host resistance to tumor growth. A crude cell membrane preparation was made by sequential hypertonic and hypotonic salt extraction of tumor cells from a fibrosarcoma induced in Syrian hamsters by simian virus 40. The membranes were chemically conjugated with dodecanoic acid. Injection (sc) of unmodified membranes ten days before transplantation of live tumor cells produced clear-cut enhancement of the tumor growth rate. In contrast, injection of lipid-conjugated membranes in a similar dose and protocol suppressed tumor growth. The lymphoid proliferative reactions to the tumor cells were consistent with

the hypothesis that unmodified membranes stimulated the production of antibody which participated in the enhancement of tumor growth, and that lipid-conjugated membranes stimulated the production of cell-mediated immunity which suppressed this growth.

1498 SERUM FACTORS MODIFYING CELL MEDIATED IMMUNITY TO RAT HEPATOMA D23 CORRELATED WITH TUMOUR GROWTH. (Eng.) Bowen, J. G. (Cancer Res. Campaign Lab., Univ. Nottingham, University Park, England); Robins, R. A.; Baldwin, R. W. *Int. J. Cancer* 15(4):640-650; 1975.

Sera from rats bearing transplanted aminoazo dye-induced hepatomas were monitored for circulating tumor-specific antigen, antibody, and immune complexes. Hepatoma D23 was induced by oral administration of 4-dimethylaminoazobenzene and was maintained by serial s.c. passage in syngeneic Wistar rats. Tumor-bearing serum was prepared by injecting rats s.c. with 5 x 10^5 viable D23 cells and bleeding at various times thereafter. Sera thus obtained were tested for tumor-specific antigen by neutralization of tumor-specific immune serum; for antibody by the indirect membrane immunofluorescence method; for immune complexes by chromatographic fractionation procedures; for blocking activity by effect on target cells in an immune lymph node cell microcytotoxicity test; and for inhibitory activity by effect on immune lymph node cells in the microcytotoxicity test. Sera from animals bearing small tumors (7-14 days after tumor implantation) contained free tumor-specific antigen, whereas immune complexes could not be detected. In comparison, sera from animals bearing large tumors (24-28 days) blocked but did not inhibit lymph node cytotoxicity, and this finding correlated with the presence of tumor-specific immune complexes in antibody excess. Animals with intermediate-sized tumors had high levels of both blocking and inhibitory activity in the serum, these effects becoming apparent when neither free antibody nor free antigen could be detected. It was concluded that circulating tumor antigen or immune complexes may play an important role in the tumor-host relationship through their interference with cell-mediated immunity.

1499 INHIBITION OF METASTASIS IN RATS IMMUNIZED WITH XENOGENIZED AUTOLOGOUS TUMOR CELLS AFTER EXCISION OF THE PRIMARY TUMOR. (Eng.) Kobayashi, H. (Hokkaido Univ. Sch. Med., Sapporo, Japan); Gotohda, E.; Hosokawa, M.; Kodama, T. *J. Natl. Cancer Inst.* 54(4):997-999; 1975.

Growth inhibition of a previously transplanted tumor by subsequent immunization with xenogenized viable tumor cells was investigated. An MCA-induced transplantable sarcoma, KMT-17, was transplanted s.c. into the right footpad of WKA rats. Xenogenized tumor X-(FV)-KMT-17, used for immunization, was obtained by serial i.p. transplantation of the tumor into Friend virus-conditioned rats. Unless the footpad was surgically removed, the transplanted KMT-17 tumor grew and metastasized to the popliteal, caudal, lumbar, and pararental lymph nodes, killing all hosts. Removal of the tumor four days after transplantation decreased the rate of metastasis to 68%. However, tumor removal and subsequent immunization with X-(FV)-KMT-17 cells allowed metastases in only 43.1% of the hosts, representing a significant reduction. A control group, immunized with xenogenized Rauscher virus-induced rat lymphoma cells, did not experience a significant reduction in metastases. The results suggested that immunization with xenogenized viable tumor cells may strongly inhibit metastasis only when combined with treatments such as surgical excision.

1500 SEQUENTIAL STUDIES OF SERUM BLOCKING ACTIVITY IN RATS BEARING CHEMICALLY INDUCED PRIMARY BOWEL TUMORS. (Eng.) Steele, G., Jr. (Wallenberg Lab., Univ. Lund, Sweden); Sjögren, H. O.; Rosengren, J. E.; Lindström, C.; Larsson, A.; Leandoer, L. *J. Natl. Cancer Inst.* 54(4):959-967; 1975.

The specific blocking activity of serial serum samples harvested from inbred WF rats bearing chemically induced primary bowel tumors was evaluated. Adenosarcomas (NG-W3, NG-W5A, NG-W5B, and NG-W9) of the colon were induced by administration per anus of N-methyl-N'-nitro-N-nitrosoguanidine (NG); adenocarcinomas (DMH-W7, DMH-W8B, DMH-W11, DMH-W26, DMH-W50, and DMABP-W8), by s.c. injections of 1,2-dimethylhydrazine-2HCl (DMH) or 3,2'-dimethyl-4-aminobiphenyl (DMABP). Each animal was checked by multiple double-contrast examinations for the occurrence and growth of bowel tumors, and a microcytotoxicity test was used for *in vitro* assay of lymphocyte cytotoxicity and serum blocking activity. Sera harvested from all tumor-bearing rats inhibited the cytotoxicity of lymph node cells from rats bearing isografts of a colon carcinoma NG-W1 against NG-W1 target cells. Serum blocking activity was found specific, and was generally demonstrated in serum obtained prior to the initial positive double-contrast examination. Results of sequential roentgenologic examinations and tests for blocking activity revealed that none of the sera could block lymphocytes specifically cytotoxic to polyoma virus-induced P-W41 tumor cells. After colon sarcomas were resected, blocking activity was not demonstrated in sera obtained on the 18-20th postoperative day. In addition, the disappearance of blocking activity correlated with possible spontaneous tumor regression. The results confirmed previous reports of antigenic cross-reactivity among bowel tumors induced by NG, DMH, and DMABP, demonstrated the specificity of the blocking activity, and revealed that such activity appeared early during primary tumor development.

1501 EFFECTS OF SERA FROM TUMOR-BEARING MICE ON MITOGEN AND ALLOGENEIC CELL STIMULATION OF NORMAL LYMPHOID CELLS. (Eng.) Whitney, R. B. (Dep. Microbiol., Univ. British Columbia, Vancouver, Canada); Levy, J. G. *J. Natl. Cancer Inst.* 54(3):733-741; 1975.

Concanavalin A (Con A) and lipopolysaccharide (LPS) stimulation of thymidine incorporation into normal

ouse spleen or lymph node cells was measured *in itro* in the presence of sera from normal or tuorous mice to determine if lymphocyte inhibition by erum factors is species or strain specific. DBA/2J ice with mammary adenocarcinoma, and three 3-methylholanthrene (MCA)-induced rhabdomyosarcomas, BALB/c ice with a melanoma or a spontaneous unclassified umor and CBA/J mice with a spontaneous rhabdomyosaroma or two MCA-induced tumors were used. Sera from ice with the spontaneous tumor and the melanoma enanced Con A stimulation of spleen cells while sera rom mice with other tumors inhibited Con A stimuation in dose-related fashions. The sera similarly ffected LPS stimulation of spleen cells. The reponses to the sera were not strain specific. The ixed lymphocyte reaction was inhibited by sera from ice with mammary adenocarcinoma and the two MCAnduced rhabdomyosarcomas in DBA/2J mice, but not by era from melanoma-bearing mice. The sera had the ame effects on Con A stimulation of normal lymph ode cells. Con A stimulation of spleen cells folowing macrophage removal was inhibited by sera from 3A/2J mice rhabdomyosarcomas and one with mammary lenocarcinoma. These same three sera inhibited Con stimulation of guinea pig spleen cells. The reilts indicate that factors present in sera from morous mice can inhibit spleen cell, lymphocyte d lymph node cell stimulation by Con A and LPS and at the factor(s) is not species or strain specific.

02 SERUM IMMUNOGLOBULIN LEVELS IN PATIENTS WITH BREAST CANCER. (Eng.) Roberts, M. (The Royal Infirmary, Edinburgh EH3 9YW, otland); Bathgate, E. M.; Stevenson, A. *Cancer* (1):221-224; 1975.

rum immunoglobulin levels IgA, IgG, and IgM have en estimated in 92 patients with breast cancer d compared to levels in 50 age-matched control tients, 40 of whom had benign disease of the east. In the patients with breast cancer, IgA s significantly raised at all stages and IgG was gnificantly reduced, but the two abnormalities re not necessarily concomitant. These findings y suggest a disturbance in the secretory immune stem, and an immunologic defect even when the ncer is localized to the breast.

03 FOWL IMMUNOGLOBULINS: QUANTITATIVE AND FUNCTIONAL RELATIONSHIP TO GENETIC RESISTCE TO MAREK'S DISEASE. (Eng.) Higgins, D. A. ornell Univ., Ithaca, N.Y.). *Diss. Abstr. Int. B* (9):4722-B; 1975.

e quantitative and functional relationship of fowl munoglobulins to genetic resistance to Marek's dise was examined in resistant and susceptible fowl. rum immunoglobulins were measured by radialimmunoffusion from 1-67-day old chickens resistant (Nne) and susceptible (P-line) to Marek's disease. ve-week-old birds were inoculated with the JM strain Marek's disease virus. Immunoglobulins were meaed and sera were examined for precipitating, vi neutralizing, and fluorescing antibodies. Antilies occurred in both lines between six and 12 days, l levels were higher in N-line birds than in P-line birds. In serum immunoglobulin levels the following stages were observed: (1) an increase in IgM and IgA 1-9 days after infection, (2) a decrease in IgM and IgA levels, and (3) until the end of experiment, IgA returned to normal and the level doubled in N-line birds. Marek's disease occured only in P-line birds, and by day 63, 1/3 of them had died. It is concluded that no significant differences occur in the immune system between resistant and susceptible fowl to Marek's disease, and that all differences are due to the immunosuppressive effects of Marek's disease virus.

1504 PREFERENTIAL HOMOLOGOUS RECOMBINATION OF H AND L CHAINS FROM MOUSE MYELOMA PROTEINS WHICH BIND DNP LIGANDS. (Eng.) Olander, J. (Jew. Hosp. St. Louis, Mo.); Little, J. R. *Immunochemistry* 12(5):383-387; 1975.

To determine the extent of preference for recombination of homologous chains in dissociated immunoglobulin populations, experiments were carried out with mixtures of heavy and light components of the 2,4-dinitrophenyl (DNP) binding murine IgA myeloma proteins, 315 and 460. Homogeneous IgA proteins were isolated from pools of sera from mice bearing plasma cell tumors MOPC-315 and MOPC-460, using immunospecific absorption onto a DNP-lysine-sepharose 4B column and elution with DNP-glycine. The proteins were iodinated by the chloramine T method for both ^{131}I and ^{125}I. Separation of the iodinated proteins was then performed by gel filtration on Sephadex G-100 in 4.5 M urea, 1 M propionic acid. In the recombination procedure, L and H chain mixtures were prepared with a slight molar excess of each L chain over the H chain. In an example, 9.40×10^{-5} mM $^{125}L_{315}$ and 7.99×10^{-5} mM $^{131}L_{460}$ were mixed with 7.59×10^{-5} mM H_{460}. The products of the reaction mixtures were separated by column chromatography on Sephadex G-150, and the distribution of the products was determined by analyses for radioactivity and for absorbance at 278 nm. The findings demonstrated that homologous chain recombinants formed preferentially, both on the basis of molar ratio of homologous to heterologous light chains and on the high specific binding to DNA-lysine-sepharose. The small magnitude observed for the preference may correlate with the similar binding specificity of the 315 and 460 proteins.

1505 CHARACTERIZATION OF TWO PLASMACYTOMA FRACTIONS AND THEIR RNA CAPABLE OF CHANGING LYMPHOCYTE SURFACE IMMUNOGLOBULINS (CELL CONVERSION). (Eng.) Katzmann, J. (Univ. Illinois Medical Center, Chicago, Ill.); Giacomoni, D.; Yakulis, V.; Heller, P. *Cell. Immunol.* 18(1):98-109; 1975.

The intracellular localization of the RNA active in cell conversion, and the nature of the plasma fraction from plasmacytomatous mice active in cell conversion was investigated. The RNA active in cell conversion was found associated with a particulate cytoplasmic fraction of the tumor that could be collected by centrifugation at 100,000 *g* for 30 min. A particulate fraction with cell-converting activity was isolated also from the plasma of tumor-

bearing animals. The plasma fraction had a buoyant density intermediate between 1.08 and 1.18 g/ml. Both active fractions migrate in the same electrophoretical region. RNA extracted from both the subcellular and plasma fraction, when analyzed on a sucrose gradient, yielded two populations of RNA molecules with cell-converting activity (14-18S and 40-50S, respectively). The 40-50S RNA was shown to be thermolabile. The RNA with cell-converting activity contained poly A stretches. The results suggest that an RNA molecule is found in a particulate fraction of mouse plasmocytoma and in the sera of mice with the tumor, and that these particles contain identical RNA molecules. During tumor growth, the plasmacytoma cells may release an RNA-containing particle that can enter normal lymphocytes, change their surface Ig, and thus lower the ability of the organism to reject the tumor.

1506 RADIOIODINATED ANTIBODY TO CARCINOEMBRYONIC ANTIGEN: BINDING TO NORMAL AND CANCEROUS HUMAN COLON *IN VITRO*. (Eng.) Coates, J. E. (Dr. W. W. Cross Cancer Inst., Edmonton, Canada); Koch, M.; Beaver, P. F.; McPherson, T. A.; Noujaim, A. A. *J. Natl. Cancer Inst.* 55(1):25-27; 1975.

Samples of tumor and normal mucosa from 32 colorectal cancer patients were examined for their capacity to bind radioiodinated antibody to carcinoembryonic antigen (anti-CEA) IgG. Tumor tissue was isolated and trimmed of normal and necrotic tissue; control samples were normal rectal biopsies from six volunteers. Twenty-three (72%) of the tumors bound significantly more antibody than the respective normal mucosa. The results indicate that radiolabeled anti-CEA may be useful in the *in vivo* localization of CEA-producing tumors and metastases in man, and may have application *in vitro* as a diagnostic marker of precancerous change in colorectal biopsies from patients at risk of developing colorectal cancer.

1507 SPONTANEOUS INTERFERON PRODUCTION AND EPSTEIN-BARR VIRUS ANTIGEN EXPRESSION IN HUMAN LYMPHOID CELL LINES. (Eng.) Adams, A. (Dept. Tumor Biology, Karolinska Inst., Stockholm 60, Sweden); Lidin, B.; Strander, H.; Cantell, K. *J. Gen. Virol.* 28(2):219-223; 1975.

Six Epstein-Barr virus (EBV)-carrying lymphoid cell lines, selected to be either extremely susceptible or very refractory to EBV superinfection, were tested for spontaneous interferon production. Interferon was titered on Daudi cells by determining its inhibitory effect on the expression of early antigen (EA) after EBV superinfection of these cells. No detectable interferon-like activity was produced by three readily superinfectable cell lines (Daudi, Raji, and NC-37). In contrast, each of three poorly superinfectable cell lines (Namalwa, Akuba, and JHTC-33) produced an antiviral substance with the general properties of interferon. These same three lines could not be induced to express EBV-specific EAs from intrinsic EBV genomes. The antiviral activities produced by the poorly infectable lines were inactivated by trypsin but not by treatment with RNase or DNase. The activity was not affected by overnight dialysis, sedimentation for two hours at 100,000 x g, prolonged exposure to pH 2, or heating at 60 C for one hour. No difference in anti-EBV titer was found if Daudi cells were treated with the antiviral preparations before or after EBV superinfection. While all preparations suppressed EBV superinfection of Daudi cells, they had little or no effect on EA expression in Raji cells. It is suggested that interferon acts as a negative control affecting a cell's susceptibility to EBV.

1508 NATURE OF THE TUMOR-ASSOCIATED DETERMINANT(S) OF CARCINOEMBRYONIC ANTIGEN. (Eng.) Hammarström, S. (Dep. Immunol., Wenner-Gren Inst., Stockholm, Sweden); Engvall, E.; Johansson, B. G.; Svensson, S.; Sundblad, G.; Goldstein, I. J. *Proc. Natl. Acad. Sci. USA* 72(4):1528-1532; 1975.

The location of the immunological determinant(s) of carcinoembryonic antigen (CEA) was investigated. CEA was purified from individual large bowel tumor metastasis to the liver, and antisera against the CEA were raised in rabbits. Carbohydrate and amino acid analyses which were carried out on the CEA showed that the carbohydrate moiety consisted of 13 structural units, excluding N-acetylneuraminic acid, and, among the various amino acids, significant amounts of cystine (three intrachain units per 10^5 gm CEA). Quantitative precipitation analysis with lectins, namely, concanavalin A, *Phaseolus vulgaris* leucoagglutinin, *Ricinus communis* lectin, and wheat germ agglutinin, revealed the presence of the following elements: terminal α-linked D-mannose and/or terminal α-linked N-acetyl-D-glucosamine. Consecutive degradations of the carbohydrate moiety by cycles of periodate oxidation, reduction, and mild hydrolysis yielded a final product containing only 15% carbohydrate, primarily N-acetylglucosamine. The degraded CEA showed no impairment of reactivity with CEA antisera. Inhibition studies in a CEA/anti-CEA precipitating system with oligosaccharides covering previously known internal structures of glycoproteins and presumably corresponding to the internal carbohydrate region of the CEA, demonstrated that none of the compounds tested was inhibitory. Further, no inhibitory effect on the binding of CEA to antibody to CEA could be demonstrated for the carbohydrate moiety prepared by hydrazinolysis or the glycopeptide fraction isolated after papain degradation of the CEA. However, reduction and alkylation of the CEA reduced the immunological activity to 3-5% and treatment with alkali completely abolished this activity without affecting the activity towards lectins. In contrast to other reports, the present results indicated that the protein moiety, not the carbohydrate moiety, of CEA carries the tumor-associated determinant(s).

1509 TUMOR-ASSOCIATED ANTIGENS. (Eng.) Holyoke, E. D. (Roswell Park Memorial Inst., 666 Elm St., Buffalo, N.Y. 14203); Chu, T. M.; Murphy, G. P. *Transplant. Proc.* 7(2):291-295; 1975.

The term, carcinoembryonic antigen (CEA) is viewed as a misnomer since the material is not exclusively,

but only relatively, associated with malignancy and the fetal state. In the present work, CEA was prepared from metastatic colon cancer of the liver by homogenization in cold water, extraction with 2M perchloric acid, dialysis, concentration by ultrafiltration, and chromatographic fractionation on Sepharose 4B and Sephadex G-200 to yield a final material judged homogeneous by immunoelectrophoresis and disc-gel electrophoresis. On analysis, the preparation appeared to be a typical glycoprotein, containing 60% carbohydrate (fucose, mannose, galactose, sialic acid and *N*-acetylglucosamine) and high concentrations of aspartic acid, glutamic acid, threonine and serine. The N-terminal sequence was determined for 30 amino acid U. Use of CEA as a clinical measurement was indicated by the finding that the measurement of serum CEA was more accurate in predicting tumor recurrence in cancer surgery patients than was notation of the presence or absence of lymph nodes. With respect to future outlook for tumor-associated antigens, it was proposed that methodological changes in the technique of radioimmunoassay are not liable to increase sensitivity and selectivity to the point necessary to provide anything approaching the screen test level of accuracy desired, and that only careful structural analysis of CEA antigenicity, study of CEA-tumor specificity, CEA formation, and CEA metabolism may be helpful.

510 ISOLATION AND CHARACTERIZATION OF A HOMOGENEOUS ISOMERIC SPECIES OF CARCINOEMBRYONIC ANTIGEN: CEA-S. (Eng.) Plow, E. F. (Scripps Clin. Res. Found., La Jolla, Calif.); Edgington, T. S. *Int. J. Cancer* 15(5):748-761; 1975.

A single homogeneous isomeric species of carcinoembryonic antigen was isolated by reference to solubility in 0.9 M perchloric acid, isoelectric focusing, molecular exclusion chromatography, ion exchange chromatography, passage through immunoabsorbants, and isopyknic density gradient ultracentrifugation. This single species of carcinoembryonic antigen,CEA-S, representing about 1.8% of the perchloric acid soluble glycoprotein of the tumor, has a sedimentation velocity of 6.6, a diffusion constant of 3.05 x 10^{-7} cm^2/sec, a mean Stokes radius of 65 Å, a density of 1.41 ml/g in cesium chloride and an estimated molecular wt of 181,000, and it is devoid of detectable A or B blood group antigens. Immunochemical studies demonstrate qualitative similarities between CEA-S and conventional carcinoembryonic antigens; however, competitive inhibition analyses demonstrate significant quantitative immunochemical differences between CEA-S and preparations of carcinoembryonic antigen. These results are consistent with the concept that CEA-S is an immunochemical isomer of carcinoembryonic antigen. The isolation of such an isomeric species lends support to the concept of speciation or isomerization of this set of tumor-associated molecules.

511 DIFFERENTIAL EXPRESSION OF EPSTEIN-BARR VIRUS EARLY AND MEMBRANE ANTIGENS AFTER INDUCTION OR SUPERINFECTION. (Eng.) Lamon, E. W. (The Medical Center, Birmingham, Ala. 35294); ...nberg, I.; Klein, G. *Cancer Biochem. Biophys.* ...1):33-38; 1974.

The expression of Epstein-Barr virus (EBV) determined membrane antigen (MA) and early antigen (EA) were followed in human lymphoblastoid cell culture lines after EBV superinfection or 5'-iododeoxyuridine (IUDR) induction of nonproducer cells. Radioiodine labeled antibody elution (RIE) using human IgG with specificity for MA or EA was used to detect these antigens in parallel with immunofluorescence. Viral capsid antigen (VCA) development was prevented by treating the superinfected cells with cytosine arabinoside (Ara C). IUDR-treated cells do not develop VCA. After 48 hr the elution peaks indicated the development of EA in both the superinfected and induced cells, increasing in quantity over the next two days. Detectable quantities of MA also appeared in the superinfected cells after 48 hr, increasing over the next two days. No MA, however, was expressed by the IUDR treated cells even after four days of exposure. EA and MA expression as detected by RIE were shown to be dependent on protein synthesis by their inhibition with puromycin. Immunofluorescence on the same cultures confirmed the findings of the RIE. It is concluded that extrinsically added EBV results in the active synthesis of MA and EA, whereas induction of the resident viral genome of these cells results in the expression of EA only.

1512 STUDIES ON A GROSS-VIRUS-INDUCED LYMPHOMA IN THE RAT. II. THE ROLE OF CELL-MEMBRANE-ASSOCIATED AND SERUM P30 ANTIGEN IN THE ANTIBODY AND CELL-MEDIATED RESPONSE. (Eng.) Knight, R. A. (Dep. Zool., Univ. Coll. London, England); Mitchison, N. A.; Shellam, G. R. *Int. J. Cancer* 15(3):417-428; 1975.

The question of the possible role of viral protein p30 as a surface antigen in virus-induced tumors was investigated. The test system involved the use of lymphomas W/Fu G-1 and W/Fu M-1, induced in Wistar Furth (W/Fu) rats by Gross and Moloney murine leukemia viruses (MuLV-G and MuLV-M, respectively). Rat antisera to the syngeneic lymphomas were cytotoxic to AKR-G-1 target cells and formed precipitates in the Ouchterlony test with disrupted Gross pseudotype of Moloney murine sarcoma virus (MuSV (MuLV-G)). This antibody cytotoxicity was inhibited by purified p30 antigen and more effectively by p30 than by purified virus, disrupted virus, extracts of virus-infected cells, or a viral envelope antigen. Gs-1-specific rabbit anti-p30 and gs-3-specific goat anti-p30 serum were cytotoxic for W/Fu G-1 target cells, showing that both the gs-1 and the gs-3 determinants were exposed on the cell surface. Spleen cells from W/Fu rats immunized with (C58NT)D Gross lymphoma cells were cytotoxic for both Moloney and Gross ^{51}Cr-labeled target cells, and both Moloney and Gross viruses inhibited the cell-mediated cytotoxicity. An equivalent increase in specific inhibition by disrupted, compared with intact, Gross and Moloney viruses suggests that common internal virion group-specific antigens are released by freeze-thawing, and that these are the cell-surface targets for cellular immunity. Moreover, the 88% inhibition observed for frozen and thawed MuLV-G suggests that internal virion proteins are the major group-specific antigens. The partial inhibition of cell-mediated cytotoxicity shown by purified p30 suggests

that this antigen is one, but not necessarily the only, internal virion antigen recognized by (C58NT)D lymphocytes. In this sytem, internal virion antigens, and in particular p30, are cytotoxic target antigens for syngeneic antibody and cell-mediated cytotoxicity. P30 is also present in the serum of rats with progressively growing lymphomas, and is the principal antigen responsible for the inhibition of cell-mediated cytotoxicity produced by these sera.

1513 IDENTIFICATION OF CONCANAVALIN A-BINDING PLASMA MEMBRANE ANTIGENS OF RAT LIVER. (Eng.) Berzins, K. (Dept. Immunology, Wenner-Gren Inst., Univ. Stockholm, Stockholm, Sweden); Blomberg, F. *FEBS Lett.* 54(2):139-143; 1975.

Attempts were made to resolve the concanavalin A (Con A)-binding plasma membrane fraction of Sprague-Dawley rat liver into individual antigens by an immunodiffusion technique. Detergent extracts of isolated plasma membranes were subjected to affinity chromatography on a Con A-Sepharose column, followed by elution and analysis of the different fractions in rocket immunoelectrophoresis. The various enzyme-active immunoprecipitates were identified by zymogram techniques. Most ATPase-active antigens and all three antigens with L-leucyl-β-naphthylamidase activity were bound to Con A. The single antigen with nonspecific esterase activity was also present in the specifically eluted material, while the catalase-active antigen appeared in the material that was not bound to Con A. The NADH-neotetrazolium reductase activity was apparently inhibited during the chromatographic procedure, as no activity could be recovered in any precipitate. Preliminary results indicate that two of the L-leucyl-β-naphthylamidase-active antigens present in the plasma membrane multienzyme complex are also present in a soluble form in the lysosomal content. These two enzymes bind to Con A, although they are not associated with phospholipids or other enzyme activities; this indicates a glycoprotein nature of the enzyme molecules.

1514 LOSS OF EPITHELIAL BLOOD GROUP ANTIGENS A AND B IN ORAL PREMALIGNANT LESIONS. (Eng.) Dabelsteen, E. (Royal Dental Coll., Copenhagen, Denmark); Roed-Petersen, B.; Pindborg, J. J. *Acta Pathol. Microbiol. Scand.* [*A*] 83(3):292-300; 1975.

Tissue from 40 oral premalignant lesions were investigated for the presence of blood group antigens A and B. The material included 18 leukoplakias, one erythroplakia, and three lichen planus, all with varying degrees of epithelial dysplasia, and 18 leukoplakias without histological evidence of impending malignancy. Thirty-eight benign keratotic oral mucosal lesions served as controls. The antigens were demonstrated by a double layer immunofluorescence technique, and the reactivity was compared, by titration, to the reactivity of adjacent normal epithelium from the same patient. All 22 lesions with dysplasia showed decreased reactivity for blood group antigen. Among the 18 leukoplakias without any signs of impending malignancy, four cases demonstrated loss of antigen reactivity. None of the 38 benign control lesions showed any change in antigen reactivity as compared to normal adjacent epithelium. It is concluded that although blood group antigen distribution does not divide dysplastic oral lesions into different groups, the cases with loss or decrease of antigen reactivity include the dysplastic lesions as well as some with no malignant change.

1515 DYNAMICS OF THE TL ANTIGENS ON THYMUS AND LEUKEMIA CELLS. (Eng.) Loor, F. (Basel Inst. Immunol., Switzerland); Block, N.; Little, J. R. *Cell Immunol.* 17(2):351-365; 1975.

A study was directed toward elucidation of the molecular mechanism of antigenic modulation (development of resistance of TL+ cells to cytolytic anti-TL antibody) and to the dynamics of the TL antigens occurring on cells other than those of the thymus. The strains of mice used as sources of thymus cells, with their TL phenotypes shown in parentheses, were: A/J (TL. 1, 2, 3) and its congenic inbred line A/TL-(TL^-); BALB/c (TL.2); DBA/2 (TL. 2); 129 (TL. 2); AKR/J (TL^-); C3H (TL^-); and C57BL/6 (TL^-). Sources of leukemic cells congenic with A/J strain mice were ASL1 (TL. 1, 2, 3) and RADA 1 (TL. 1, 2, 3). Anti-TL antisera were prepared by immunization of inbred A/TL-mice with TL+ ASL1 leukemic cells. TL antigens on surfaces of leukemic cells and thymus cells were detected by indirect immunofluorescence using labeled rabbit anti-mouse Ig antibody. TL antigens could be capped by anti-TL antibodies, although no detectable microprecipitation (spotting) occurred. Thus, TL antigens were found on the majority of thymus cells from TL^+ strains of mice and on virtually all RADA 1 leukemia cells. Further, they were not detected in a strain (A/TL^-) congenic with A/J, on other TL^- strains, or on hybrids between TL^- strains, thus confirming a lack of complementation of TL deficiencies. Conversely, hybrids between a TL^+ and a TL^- strain were found to be TL^+, consistent with simple autosomal dominance of TL expression on thymocytes. The immunofluorescence findings were in accord with the classification of strains as TL^+ and TL^- provided previously by cytotoxicity testing and semiquantitative adsorption studies. Thymus cells lost TL antigens spontaneously during culture, while leukemic cells did not. Leukemic cells slowly cap and become depleted of TL antigens when cultured in the presence of anti-TL bodies. Leukemic cells, in contrast to TL^+ thymocytes, re-express their TL antigens after capping when cultures are transferred into noncapping conditions. Lack of re-expression of TL after capping of thymocytes was not due to damage to thymus cell metabolism, since H-2 antigens could be re-expressed (as detected by anti-H-2 antibody).

1516 HUMAN MENINGIOMA ANTIGENS. (Eng.) Winters, W. D. (Sch. Med., Univ. California, Los Angeles); Rich, J. R. *Int. J. Cancer* 15(5):815-822; 1975.

Soluble antigens extracted and purified from surgical specimens of human meningiomas (MSA) were used

to test for precipitating antibodies in sera from patients with various histologic types of brain tumors, non-neural solid tumors and from normal donors. Blind studies by immunodiffusion showed that 63% (15/24) of meningioma patients, 53% (9/17) of glioma patients and 17% (5/29) of patients with various other brain neoplasms had antibodies that reacted with two of three meningioma-associated antigens. Sera from normal donors and patients with non-neural solid neoplasms reacted to a limited extent (7/118) with another of these tumor-associated antigens. Cross-reaction and absorption studies revealed that the three meningioma-associated antigens were detecting different antibodies. None of the antigens was related to HLA antigens or to the human non-neurotropic viruses used. These studies suggest the presence of at least three different antibody-antigen reaction systems, or at least three different soluble antigens in the meningioma antigenic preparations. This occurrence might suggest common etiologic agents in these neural tumors.

1517 HUMAN MELANOMA AND LEUKEMIA ASSOCIATED ANTIGENS DEFINED BY NONHUMAN PRIMATE ANTISERA. (Eng.) Seigler, H. F. (Yerkes Regional Primate Center, Emory Univ., Atlanta, Ga. 30322); Metzgar, R. S.; Mohanakumar, T.; Stuhlmiller, G. M. *Fed. Proc.* 34(8):1642-1646; 1975.

Advantage was taken of the antigenic similarities between man and nonhuman primates for blunting the immune response of nonhuman primates to human species-specific antigens. Response to allogeneic HL-A antigens could then be managed by *in vitro* absorption. ABO compatible chimpanzees were selected on the basis of alloantigens shared with the HL-A phenotype of the immunizing tumor donor. Absorption of chimpanzee antihuman melanoma serum with normal lymphocytes and skin fibroblasts had little effect on cytotoxicity against 14 melanoma target cell lines. Absorption with melanoma cells removed all cytotoxicity against six different melanoma cell lines. This chimpanzee antiserum, therefore, did not detect a tumor-associated antigen individual to the tumor host, but did suggest the existence of a cross-reacting tumor associated antigen for melanoma. Antiserum absorbed with normal tissues lysed fetal target cells; that absorbed with normal and fetal cells removed antifetal activity and left antitumor activity antiserum absorbed with normal and melanoma cells removed both antitumor and antifetal activity. Antisera were also prepared in monkeys and chimpanzees against leukemia cells from human patients with chronic lymphocytic leukemia (CLL), chronic granulocytic leukemia (CGL), and acute myelogenous leukemia (AML). After absorption with human RBC and peripheral WBC from normal donors, antisera to human CLL cells were cytotoxic to cells from all CLL and acute lymphocytic leukemia (ALL) patients, and nonreative with normal, CGL or AML cells. Similarly absorbed antisera prepared against CGL or AML cells were nonreactive with normal, CLL or ALL cells but revealed a broad spectrum of antigens associated with myelogenous leukemia patients. By contrast, rabbit antisera against cells of different classes of leukemia were unable to differentiate between the classes. The primate antisera has proved to be a valuable diagnostic agent.

1518 QUANTITATIVE IMMUNOFLUORESCENCE STUDIES OF THE TUMOR ANTIGEN-BEARING CELL IN GIANT CELL TUMOR OF BONE AND OSTEOGENIC SARCOMA. (Eng.) Byers, V. S. (Dept. Dermatology, Univ. California San Francisco, San Francisco, Calif. 94143); Levin, A. S.; Johnston, J. O.; Hackett, A. J. *Cancer Res.* 35(9):2520-2531; 1975.

Studies were conducted to determine whether any of the cellular components of a giant cell tumor of the bone possessed a tumor-associated antigen, and, if so, which cells carried the antigen. Whether the antigen-bearing cells could be established in culture was also determined. Tumor-associated antigen was found by reacting sera from two pateints with giant cell tumors of bone with cells derived from their tumors, using autologous serum as an intermediate reactant and fluorescein-conjugated goat anti-human IgG as the final reactant. Approximately 40% of the plump, spindle-shaped cells that formed the background stroma of these tumors possessed the antigen; however, it was not present on giant cells. Fluorescence was much greater than that on similarly stained cells from four osteogenic sarcomas, suggesting that the antigenic density on cells from giant cell tumor was greater than that on cells from osteogenic sarcoma. Antibodies in sera from giant cell tumor patients and osteogenic sarcoma patients showed specific cross-reactivity. Stromal cells of giant cell tumors were established in culture and retained tumor-associated antigen, whereas giant cells failed to divide and detached from the flask within two wk. Intensity of fluorescence (antigenic density) decreased with progressive passage levels, but a larger percentage of cells showed fluorescence. At the tenth passage, all cells bore tumor-associated antigen. Cultured cells that were injected s.c. into mice formed progressively growing nodules, the cells of which were morphologically indistinguishable from stromal cells of the original tumor; all cells retained tumor-associated antigen, but antigenic density had decreased to about one-seventh of the value found originally. No giant cells were present in the nodules. It is suggested that at least some giant cell tumors of bone represent a variant of the same pathological process that occurs in osteogenic sarcoma and that the different clinical course is a result of host immune response.

1519 INHIBITION OF LEUKOCYTE MIGRATION BY TUMOR-ASSOCIATED ANTIGENS IN SOLUBLE EXTRACTS OF HUMAN MALIGNANT MELANOMA. (Eng.) McCoy, J. L. (Litton Bionetics, Inc., Kensington, Md.); Jerome, L. F.; Dean, J. H.; Perlin, E.; Oldham, R. K.; Char, D. H.; Cohen, M. H.; Felix, E. L.; Herberman, R. B. *J. Natl. Cancer Inst.* 55(1):19-23; 1975.

Direct leukocyte migration inhibition (LMI) assays were performed to determine whether cell-mediated immune reactions could be detected in response to tumor-associated antigens of human melanoma. The antigens were 3 M KCl-soluble extracts of different fresh melanomas, other cancers, and benign nevus tissue. A total of 48 of the 79 (61%) blood samples from melanoma patients (64 patients) reacted with extracts of melanoma tissue. Since the sub-

jects were usually tested with two or three extracts, 57 of 134 (42%) tests with melanoma patients' leukocytes were inhibited by KCl extracts of melanoma tissue, whereas only 3 of 50 (6%) tests with leukocytes of normal donors and 4 of 27 (15%) with patients having other cancers gave positive results. No positive reactions were obtained when 13 melanoma patients were tested with a 3 M KCl extract of benign nevus tissue. Likewise, only 2 of 26 (8%) positive tests were obtained from melanoma patients tested with extracts of other cancers. Individuals in all stages of disease had similar incidences of positive reactions to the soluble melanoma extracts, except for patients with stage-1 disease who exhibited a somewhat higher incidence of reactivity. The highest incidence of reactivity was observed in patients before surgical resection of the tumor, and somewhat decreased reactivity was seen 0-14 days post-surgery. The results indicate that the direct LMI assay may be used to measure cell immune reactivity against melanoma-associated antigens. Since many of the positive results were obtained with allogeneic extracts, the results also indicate that different melanomas possess common antigens.

1520 SOLUBLE TUMOUR-SPECIFIC ANTIGEN AND ITS RELATIONSHIP TO TUMOUR GROWTH. (Eng.) Thomson, D. M. P. (Montreal General Hosp. Res. Inst., McGill Univ., Montreal 109, Quebec, Canada). *Int. J. Cancer* 15(6):1016-1029; 1975.

Hooded rats bearing a syngeneic methylcholanthrene-induced tumor were evaluated for extent of *in vivo* host immunity and this was correlated by *in vitro* techniques with the levels of circulating tumor antigen and specific antibody. Early tumor growth was associated with detectable immunity, as measured by the capacity of the animal to reject a second direct challenge of the same tumor at a remote site. Radioimmunoassay for circulating tumor antigen and indirect membrane immunofluorescence for antitumor antibody did not detect either component at this stage. Animals with advanced tumors lost immunity as detected by direct tumor challenge, and this closely coincided with the appearance of rising levels of circulating soluble tumor antigen. Although the host possessed the immunologic ability to react against its own neoplasm, this ability was insufficient to produce tumor rejection. Active immunotherapy initiated at the time of, or up to ten days after, im challenge with tumor 1.5×10^4 cells, increased tumor immunity sufficiently for tumor growth to be prevented. Successful immunization was associated with the early appearance (16 days) of measurable levels of antitumor antibody and absence of circulating antigen. It is concluded that soluble tumor antigen present in the local microenvironment of the tumor in the early stages of tumor growth interferes with the ability of immune cells to cause tumor rejection. As the tumor progressively grows, sufficient soluble antigen is produced and released systemically to suppress the effector arm of the host's tumor immune response at distant sites. The levels of circulating soluble tumor antigen attained may be of critical importance in the suppression of rejection responses that prevent metastasis.

1521 SURFACE ANTIGENS ON CULTURED MALIGNANT MELANOMA CELLS AS DETECTED BY A MEMBRANE IMMUNOFLUORESCENCE METHOD WITH HUMAN SERA. LACK OF TUMOR-SPECIFIC REACTIONS ON MELANOMA LINES. (Eng.) The, T. H. (Dept. of Internal Medicine, Univ. of Groningen, Groningen, The Netherlands); Huiges, H. A.; Koops, H. S.; Lamberts, H. B.; Nieweg, H. O. *Ann. N.Y. Acad. Sci.* 254:528-540; 1975.

A membrane immunofluorescence (MIF) method was used to detect circulating antibodies in sera from melanoma patients against surface antigens of cultured melanoma cells. The cells used consisted of short-term cultures (1-10 wk) or long-term lines (at least 60 generations). Serum from a melanoma patient, containing anti-HLA antibodies, showed a fine granular fluorescence in an MIF test with melanoma cells. After the treatment of such serum with pooled tonsillar lymphocytes to remove the anti-HLA antibodies, the fine staining disappeared, while a coarse patchy reaction remained. Further absorption with melanoma line suspensions removed all antibody activity. Tests on early passages of melanoma cell cultures were positive in 14 of 21 cases (66%) if tested with autologous sera, whereas only 32 of 128 cases (25%) were positive with allogeneic melanoma patients' sera. Normal sera showed weak reactions in 4 of 24 cases (16%). Control experiments with fibroblasts were negative. These findings indicate that tumor-associated surface antigens are maintained during short-term cultures and confirm earlier reports on the existence of individual-specific antigens on melanoma cells. Cells from long-term cultures of melanoma lines reacted more frequently and also more strongly with allogeneic melanoma patients' sera. However, these reactions were not melanoma-specific, because no differences were found in the results with sera obtained from 17 melanomas, seven skin carcinomas other than melanoma, and eight digestive tract carcinomas. A lack of tumor specificity was shown by cross reactivity of positive melanoma patients' sera in MIF tests using cervical cancer and urinary bladder carcinoma lines. Quantitative differences in antigen expression were detected between the melanomas and other cell lines by absorption experiments, but there was again no evidence for the existence of tumor-specific surface antigens on the melanoma cells. Furthermore, melanoma cells from fresh biopsies were less effective in absorption than were cells from melanoma lines, and cells from 10-wk-old fetuses also were active. It is concluded that the broad cross reactivity of melanoma cells acquired during culture makes them unsuitable for detecting tumor-specific antibody reactions with the MIF technique.

1522 AUTOANTIBODIES IN PATIENTS WITH CARCINOMA OF THE BREAST: CORRELATION WITH PROGNOSIS. (Eng.) Wasserman, J. (Cent. Microbiol. Lab., Stockholm Cty. Counc., Sweden); Glass, U.; Blomgren, H. *Clin. Exp. Immunol.* 19(3):417-422; 1975.

An indirect immunofluorescence technique was used to test sera from 100 patients with breast cancer and

rom 75 controls for antinuclear antibodies (ANA), nooth muscle antibodies (SMA), glomerular antibodies GA) and mitochondrial antibodies (MA). There was no ifference in the incidence of one antibody in paients and controls, but only 39% of the patients nd 61% of the controls demonstrated no antibodies. one of the controls demonstrated more than one antiody whereas 20% of the patients did. Seventy-nine f the 100 patients remained free of recurrence; of hese, 35 had no antibodies and 11 had more than one, hereas only four of 21 patients with local recurrnce or distant metastasis had no antibody levels nd nine had more than one type of antibody. There as no difference in the incidence of GA in the two roups, and MA were found in only three of the caner patients. The incidence of more than one antiody was found to be higher in patients with recurrnces than in patients in remission. The results inicate that the early presence of certain antibodies n patients with breast cancer may be a bad prognosic sign.

523 HL-A ANTIGENS IN CARCINOMA OF THE BREAST, OVARIUM, CERVIX AND ENDOMETRIUM: POSSILE ASSOCIATION OF HAPLOTYPE HL-A 10-W18 WITH CARINOMA OF THE BREAST. (Eng.) Bertrams, J. (Laboatorien des Elisabeth-Krankenhauses D-4300 Essen oltkestr. 61 Federal Republic of Germany); Thraenart, O.; Feldmann, U.; Kuwert, E. *Z. Krebsforsch.* 3(3):219-222; 1975.

ossible correlations between tumor diseases of man nd HL-A antigens were investigated. A total of 63 Caucasian cancer patients (60 breast cancers, 4 cancers of the cervix uteri, 19 cancers of the ndometrium and 20 cancers of the ovary) and 1,000 ealthy unrelated subjects of similar ethnic and eographic background were tissue-typed with 72 seective HL-A antisera defining HL-A specificities n a microcytotoxicity test. The comparisons of antigen frequencies in the two groups yielded no outtandingly significant deviations for cancer of the ervix, endometrium and ovary. When the present ata for cancer of the breast were combined with hose published by four other groups of investigaors, the results of a statistical analysis suggesed that there might be an association of the disase with the antigens HL-A10 and W18 or the haploype HL-A10-W18. Interpreted in terms of risk of eveloping carcinoma of the breast, the analysis ndicates that healthy persons bearing the antigens L-A10 and W18 have a 40% higher risk than HL-A10nd W18-negative individuals.

524 BURKITT'S LYMPHOMA AND HL-A ANTIGENS. (Eng.) Dick, H. M. (R. Infirm., Glasgow, cotland); Steel, C. M.; Levin, A. G.; Henderson, N. *issue Antigens* 5(1):52-58; 1975.

microcytotoxicity technique was used to determine he incidence of HL-A antigen in 33 Kenyan Burkitt's ymphoma patients (14 females and 19 males) and 37 ontrols (15 females and 22 males). There was no ifference in the incidence of HL-A antigen between ontrols and Burkitt's lymphoma patients. The authors report difficulty in HL-A identification using HL-A7 and "W19" complex Caucasoid serum antibodies. The authors suggest further studies on the inheritance of HL-A antigen in the families of Burkitt's lymphoma patients.

1525 HL-A AND BURKITT'S TUMOUR -- A STUDY IN UGANDA. (Eng.) Bodmer, J. G. (Genet. Lab., Oxford, England); Bodmer, W. F.; Ziegler, J.; Magrath, I. T. *Tissue Antigens* 5(1):59-62; 1975.

Serum samples from 31 Burkitt's lymphoma patients (18 males and 13 females) and from 48 controls were HL-A typed using the cytotoxic fluorochromatic assay to investigate whether the frequency of occurrence of the antigen might be associated with susceptibility to the disease. The frequencies of HL-A antigen in Burkitt's lymphoma patients was not different from that of controls. The authors conclude that the study was too small to detect a loose association of HL-A antigen with Burkitt's lymphoma.

1526 ANTIGENS ASSOCIATED WITH CHEMICALLY INDUCED INTESTINAL CARCINOMAS OF RATS. (Eng.) Martin, F. (Laboratory of Experimental Medicine, Faculte de Medecine, Bd. Jeanne d'Arc, 21000 Dijon, France); Martin, M. S.; Bordes, M.; Knobel, S. *Int. J. Cancer* 15(1):144-151; 1975.

Rabbits were immunized with extracts of primary or grafted intestinal adenocarcinomas induced by carcinogenic drugs in inbred rats in an effort to determine whether antisera obtained from these rabbits could detect tumor-associated antigens. Serially transplantable tumor lines were obtained from a duodenal adenocarcinoma induced by N-methyl N'-nitro N-nitrosoguanidine in a Lewis strain rat and five colonic adenocarcinomas induced by 1,2-dimethylhydrazine in BD-IX rats. Twenty-one rabbits were immunized with saline or perchloric acid extracts of primary or grafted intestinal tumors. The sera were absorbed with lyophilized rat plasma (100 mg/ml), lyophilized saline extracts of colon from BD-IX strain rats (100 mg/ml), and lyophilized saline extracts of small intestine from Lewis strain rats (100 mg/ml). After absorption, the antisera were able to recognize three tumor-associated antigens. Two of them were glycoproteins, which are present in cancer cells and also in trace amounts in mucous cells of the normal digestive tract. The third antigen was not detectable in the normal digestive system, but was present in normal spleen. This antigen was detected by immunofluorescene in polymorphonuclear cells infiltrating the tumor, but not in the cancer cells. None of the three antigens cross-reacted with human carcinoembryonic antigen, or human or rat alphafetoprotein. One of the glycoprotein antigens reacted with saliva from individuals of blood group A, indicating that it is immunologically related to the human blood group A substance. It is suggested that the lack of cancer-specificity of the antigens does not preclude the existence of tumor-specific antigens in the experimental intestinal carcinoma; instead, it establishes the inadequacy of the heteroimmunization procedures generally used to detect these antigens.

1527 NEOANTIGENS ON CHEMICALLY TRANSFORMED CLONED C3H MOUSE EMBRYO CELLS. (Eng.) Embleton, M. J. (The Medical Sch., Univ. of Wisconsin, Madison, Wisc. 53706); Heidelberger, C. *Cancer Res.* 35(8):2049-2055; 1975.

Using an *in vitro* cytotoxicity test for cell-mediated immunity and a membrane immunofluorescence test, the appearance of new antigens was detected on 3-methylcholanthrene- or 7,12-dimethylbenz[a]anthracene-transformed cloned C3H mouse embryo cells. These antigens were recognized by specifically immunized syngeneic mice and were individually unique for each of eight chemically transformed cell lines tested, all derived from the same control clone. Very few cross reactions were seen between lymphoid cells or antibody from mice immunized against a given cell line and target cells of other cell lines. New antigens could not be detected on two spontaneously transformed lines. Lymphoid cells from multiparous pregnant or embryo-immunized mice were used to search for fetal antigens on control and transformed cells. Fetal antigens were detected on seven of the chemically transformed cell lines and one spontaneous transformant, but not on nontransformed control cells. It is concluded that individually specific new antigens are characteristic of chemically transformed cells, but the expression of fetal antigens may be a more common feature of transformed cells in general.

1528 PRODUCTION OF MIGRATION INHIBITION FACTOR (MIF) BY HUMAN ESTABLISHED B TYPE CELL LINES DERIVED FROM NORMAL AND MALIGNANT TISSUES: STUDIES OF SOME FACTORS AFFECTING MIF RELEASE. (Eng.) Florentin, I. (Institut de Cancérologie et d'Immunogénétique [INSERM et Association Claude-Bernard] Hôpital Paul-Brousse, 14-16, avenue Paul-Vaillant-Couturier, 94800-Villejuif, France); Bruley, M.; Belpomme, D. *Cell. Immunol.* 17(1):285-294; 1975.

Factors associated with the production and release of migration inhibition factor (MIF), a lymphokine molecule, were studied in 25 permanent human lymphoid cell lines initiated from peripheral blood WBC of normal donors or from patients with hemopoietic diseases. The cells were characterized as follows: B lymphocytes and monocytes by EAC rosette formation, T cells by spontaneous rosette formation when the cells were mixed with sheep RBC, and immunoglobulins (Ig) by immunofluorescent staining. Thirty-seven to ninety percent of the cells were EAC rosette-forming and 50-80% of the cells had surface Ig. No spontaneous rosettes were detected. All cell lines released MIF into culture fluid and no significant difference was observed among lines of varying origin. Kinetic studies revealed that spontaneous MIF production was related to the establishment phenomenon. The lag observed before MIF activity was proportional to the size of the inoculum and appeared when the cell density in culture reached 10^6 cells/ml. The age of the cell lines ranged from one mo to five yr, indicating that the ability to secrete MIF is a long-lived and stable process. MIF detection in established cultures depends on various *in vitro* parameters including the cell growth pattern and the target cell used for the migration inhibition test. The significance of MIF production by permanent cell lines bearing B surface antigens is also discussed.

1529 QUANTITATION OF T AND B LYMPHOCYTES AND CELLULAR IMMUNE FUNCTION IN HODGKIN'S DISEASE. (Eng.) Bobrove, A. M. (Stanford Univ. Sch. of Medicine, Stanford, Calif. 94305); Fuks, Z.; Strober, S.; Kaplan, H. S. *Cancer* 36(1):169-179; 1975.

Peripheral blood T and B lymphocytes were quantitated in 42 patients with untreated Hodgkin's disease and the results were compared with the response to phytohemagglutinin (PHA) stimulation and delayed hypersensitivity skin testing. T lymphocytes were identified by an *in vitro* cytotoxicity assay employing a specific anti-T-cell serum and by spontaneous rosette formation with sheep erythrocytes (E rosettes). The percentage of T cells in the patients was similar to that of normal subjects as judged by the cytotoxicity assay (65 to 90%). In addition, absolute T-lymphocyte counts were normal in 63% of the patients and were generally reduced only in those with lymphopenia. The percentage of T lymphocytes determined by the E-rosette assay was similar to that determined by the cytotoxicity assay in normal controls, but was significantly lower than that determined by the cytotoxicity assay in the patients. The decreased response to PHA stimulation in the patients was directly correlated with the decrease in E-rosette formation. These findings suggest that T lymphocytes in the peripheral blood are not generally diminished in untreated Hodgkin's disease. However, a proportion of these cells exhibits altered surface interactions that may account for some aspects of their impaired immunologic function.

1530 *IN VITRO* STUDIES OF AXILLARY LYMPH NODE CELLS IN PATIENTS WITH BREAST CANCER. *(Eng.)* Tsakraklides, E. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Tsakraklides, V.; Ashikari, H.; Rosen, P. P.; Siegal, F. P.; Robbins, G. F.; Good, R. A. *J. Natl. Cancer Inst.* 54(3):549-556; 1975.

One hundred and seventy axillary lymph nodes obtained from 81 patients who had radical mastectomies were tested for the presence of T and B cells using immunofluorescence microscopy and the sheep RBC (SRBC) rosette technique in order to determine the role of local nodes in the immunology of breast cancer. Sixty-three lymph nodes were tested for functional capacity by response to phytohemagglutinin (PHA), 10 μg, 20 μg, and 30 mg/100 μl) and ten nodes were studied for concanavalin A (50, 100, and 150 μg/ml) response. From 32-80% of the lymph node cells (LNC) were T cells. B cells characterized by the presence of surface immunoglobulins made up 14-61% of the LNC; B cells characterized by C_3 receptors made up 8-54%, and B cells characterized by IgG-specific (Fc) receptors ranged from 10-45% of the LNC. All of the LNC which did not bind to SRBC showed the presence of surface immunoglobulins, but only 2/3 were positive for C_3 and Fc receptors. Stimulation of ^{14}C-thymidine

ncorporation into lymphocytes by PHA and Concanavaln A was greater in material from younger patients han that from older ones, greatest in lymph nodes ith lymphocyte predominance and lowest in nodes ith lymphocyte depletion (germinal center predomnance) and greater in nodes from patients without etastatic disease than in nodes from patients with etastases. Lymph nodes from patients with metasatic disease and from patients over 60 yr old had ower proportions of T cells and higher proportions f B cells than nodes from patients without metasases or those who were 45 yr of age or younger. ymph nodes with germinal center predominance had ow proportions of T cells, whereas lymph nodes ith lymphocytic predominance had high proportions f cells. The authors suggest that this inforation may be useful in determining the immunologial significance of regional lymph nodes in breast ancer, but that it does not give a direct answer o the question of lymph node preservation or exision in breast cancer surgery.

531 SURFACE MARKERS ON HUMAN B AND T LYMPHOCYTES. VII. ROSETTE FORMATION BETWEEN ERIPHERAL T LYMPHOCYTES AND LYMPHOBLASTOID B-CELL INES. (Eng.) Jondal, M. (Department of Tumor iology, Karolinska Institutet, 104 01 Stockholm 0, Sweden); Klein, E.; Yefenof, E. *Scand. J. Immol.* 4(3):259-266; 1975.

he capacity of peripheral T lymphocytes to form roettes with human lymphoblastoid B-cell lines was tudied. The lines were derived from patients with eukemia, Burkitt's lymphoma, or infectious mononuleosis and from normal persons. Of 16 reacting ines, eight showed 75-95% rosette-forming cells nd eight showed 5-15%. Of lines reacting at the igh level, 5 of 8 were IgG lines; of the low rective lines, 7 of 8 were IgM lines. The expression f surface immunoglobulin on some lines varied durng the investigation, and some lines that periodially lost their surface immunoglobulin were still ble to form rosettes. Four lymphoblastoid T-cell ines, together with human myeloma, myelocytic, and arcinoma lines and a mouse mastocytoma line were nreactive. The phenomenon was temperature-sensiive, predominating at 4 C. The binding of T lymphoytes to B-cell lines was weak; most attachments ould be broken by strong agitation. T-lymphocytes, last-transformed by stimulation with mitomycin Created allogeneic lymphocytes, retained the capacity o form rosettes, even at 37 C. Unstimulated T cells ound less readily to B-cells blast-transformed with okeweed mitogen. Even though rosettes formed at 4 were stable for several hours at 37 C, no T-cellediated cytotoxicity could be detected during overight incubation. Rosette formation was decreased t pH 5 or pH 10, or following trypsinization of the ymphocytes or the B-cell lines, while neuraminidase reatment of the lymphocytes increased the rosette ormation. Anti-Ig, anti-β_2-microglobulin, or anti sera did not influence the rosette formation. The esults of varying these factors did not clearly inicate the surface structures responsible for the inding. The T-lymphocyte characteristic may be of ome importance in the classification of human lymhoid malignancies.

1532 SURFACE MARKERS ON HUMAN B AND T LYMPHOCYTES. VI. CYTOTOXICITY AGAINST CELL LINES AS A FUNCTIONAL MARKER FOR LYMPHOCYTE SUBPOPULATIONS. (Eng.) Jondal, M. (Dep. Tumor Biol. Karolinska Inst., Stockholm, Sweden); Pross, H. *Int. J. Cancer* 15(4):596-605; 1975.

The spontaneous lymphocyte-mediated cytotoxicity (SLMC) of lymphocytes from normal donors was tested against 19 different cell lines. The lymphocytes were purified from heparinized whole blood by buoyant density centrifugation and iron carbonyl powder treatment. Removal of lymphocytes with Fc and C3 receptors was accomplished in certain tests by buoyant density centrifugation of Fc and C3 rosettes. Cell lines used for target cells were labeled with ^{51}Cr and maintained as stationary suspension cultures. All cell lines tested were found to be susceptible to the lytic capacity of lymphocytes from normal donors. The cytotoxicity was dose-dependent on the number of lymphocytes, decreased by trypsin pretreatment, NAF and/or EDTA addition, and unaffected by neuraminidase, different media, or purification factors. SLMC was found to be mediated by a minor subpopulation of lymphocytes, as evidenced by the observation that removal of either the Fc or C3 lymphocytes severely decreased the cytotoxicity. Addition of high concentrations of concanavalin A (Con A) blocked the cytotoxicity of unfractionated cells, while relatively lower concentrations induced a cytotoxic response in noncytotoxic, C3-depleted cells. The results supported the view that SLMC is a non-specific non-immunological phenomenon occurring, to varying degrees, in all combinations of normal lymphocytes and different cell lines. SLMC appeared to be a functional characteristic of Fc/C3-binding cells, whereas Con A-induced cytotoxicity of non-Fc/C3-binding cells appeared to be a T-cell function.

1533 SPECIFIC LYMPHOCYTE-ACTIVATING DETERMINANTS EXPRESSED ON MOUSE MACROPHAGES. (Eng.) Schirrmacher, V. (London Hosp. Med. Coll., England); Peña-Martinez, J.; Festenstein, H. *Nature* 255(5504):155-156; 1975.

Previous cell fractionation studies revealed that lymphocyte-activating determinants (LADs) of the multigenic major (H-2) histocompatibility system (MHS) are expressed on both B and T lymphocytes, whereas LADs of the unigenic (non-H-2) *M* locus are preferentially expressed on B lymphocytes. Lymphocytes from BABL/c, (BALB/c x DBA/2)F_1, and DBA/2 mice were cultured with allogeneic or semiallogeneic macrophages. Parental lymphocytes proliferated well in response to F_1 macrophages differing either at the *M* locus or at the H-2 complex, but F_1 lymphocytes did not respond to parental macrophages. Results obtained with cells from the *H*-2^d identical mice DBA/2 and BALB/c showed stimulation of BALB/c lymphocytes by DBA/2 macrophages but no stimulation of DBA/2 lymphocytes by BALB/c macrophages. Unilateral stimulation of BALB/c by DBA/2 has been reported with lymphocytes as stimulator cells and attributed to the LAD coded for the *Mls*^{a} allele at the *M* locus. These experiments indicate that LADs of the H-2 complex as well as LADs of the *M* locus are

apparently expressed on macrophages. Similar specific responses of mouse lymphocytes were observed in cultures of highly purified allogeneic or semiallogeneic macrophages.

1534 THE IMMUNOLOGIC RESPONSIVENESS OF REGIONAL LYMPHOCYTES IN EXPERIMENTAL CANCER. (Eng.) Goldfarb, P. M. (Albert Einstein Coll. Med., Bronx, N. Y.); Hardy, M. A. *Cancer* 35(3):778-783; 1975.

The relationship between the immunologic reactivity of regional lymph node (RN) lymphocytes and distant (DN) and central (CN, splenic tissue) lymphocytes was investigated in relation to the initiation and progression of mammary adenocarcinoma in mice. Three groups of 100 female C_3H/HeJ mice, 6-9 weeks old, were injected s.c. with 10^6 viable murine mammary adenocarcinoma cells, 10^3 tumor cells, or Millipore chambers containing 10^6 viable cells. Four groups of five mice each were bled and sacrificed on days 3, 7, 14, 21, and 28. Controls consisted of unsensitized lymphocytes from axillary lymph nodes, inguinal lymph, and spleens from the same group of mice not injected. Lymphocytes from different areas of the three groups and controls were cultured with phytohemagglutinin (PHA-P), autologous tumor, or normal C_3H/HeJ breast tissue. RN lymphocytes, (those which were adjacent to growing tumor or to Millipore chambers), showed the earliest peak response to PHA-P and autologous tumor (seven days after tumor inoculation), while the CN lymphocytes and DN lymphocytes were delayed (21 days for response to PHA-P and 14 days for response to autologous tumor); the latter was especially true in mice receiving Millipore chambers containing 10^6 viable cells. These studies demonstrate that RN lymphocytes are the first to be maximally sensitized to tumor antigens during the initiation of tumor growth and are involved in tumor recognition. The development of central immunity appears to be the result of dissemination of immunogenic tumor products initially mediated by the RN.

1535 THE CHRONIC LYMPHATIC LEUKEMIA LYMPHOCYTE: CORRELATION OF FUNCTIONAL, METABOLIC, AND SURFACE IMMUNOGLOBULIN CHARACTERISTICS. (Eng.) Sagone, A. L., Jr. (Ohio State Univ., Coll. Med., Columbus, Ohio); Murphy, S. G. *Cell. Immunol.* 18(1): 1-8; 1975.

The response of chronic lymphatic leukemia lymphocytes to nonspecific mitogens was studied in order to correlate the functional capacity, glucose metabolism, and immunoglobulin characteristics of these cells. Phytohemagglutinin (PHA) was added to lymphocyte cultures at 25 μl/10^6 cells; pokeweed mitogen was added at 10 μl/ml. [$1-^{14}C$]glucose or [$6-^{14}C$] glucose (each 2 μCi) were added and $^{14}CO_2$ produced by the cells was measured after 68 hr. A majority of patients (12 of 14) had lymphocytes with impaired transformation to both PHA and pokeweed mitogens. These cells also had impaired glucose metabolism in unstimulated cultures and lacked the striking increase in glucose metabolism in response to mitogens which is characteristic of normal lymphocytes. Most of these lymphocytes had IgM surface immunoglobulins. Two patients had lymphocytes with normal lymphoblastic transformation to PHA and impaired transformation to pokeweed, suggesting cells of T origin. The glucose metabolism of these lymphocytes were less impaired in unstimulated cultures than those of the other patients, and had a striking increment in glucose metabolism in response to PHA similar to normal lymphocytes. Unexpectedly, these lymphocytes were found to have IgG on their surface, suggesting cells of B origin. These results indicate that there may be two groups of chronic lymphatic leukemia patients with clinically similar disease in whom the functional and metabolic characteristics of the lymphocytes are different and that the surface immunoglobulin characteristic of lymphocytes may not always predict their functional characteristic.

1536 *IN VITRO* "EDUCATION" ON AUTOLOGOUS HUMAN SARCOMA GENERATES NON-SPECIFIC KILLER CELLS. (Eng.) Martin-Chandon, M. R. (Dep. Tumor Biol., Karolinska Inst., Stockholm, Sweden); Vanky, F.; Carnaud, C.; Klein, E. *Int. J. Cancer* 15(2):342-350; 1975.

Peripheral blood lymphocytes from three sarcoma patients were exposed to autologous tumor-cell monolayers to determine whether *in vitro* sensitization (education) would occur in human autologous tumor systems. Cell-mediated cytotoxicity of the lymphocytes was measured following 3-5 days education using either fibroblasts or tumor target cells. In one patient, lymphocytes educated on autologous tumor cells were strongly cytotoxic to both fibroblasts and tumor cells. Freshly drawn lymphocytes from the tumor patients and from a healthy donor, and fibroblast-educated lymphocytes were all without effect. In a second patient, the results were similar. In the third patient, freshly drawn and educated lymphocytes were assayed against autologous and allogeneic target cells. The freshly drawn lymphocytes were cytotoxic to autologous and allogeneic sarcoma cells, weekly cytotoxic to autologous fibroblasts and inactive against allogeneic fibroblasts. The educated lymphocytes from this patient were cytotoxic to all four target-cell types. These non-specific killer cells were not produced when the lymphocytes were co-cultivated along with autologous fibroblasts. Addition of autologous serum during education inhibited the development of the killer cells, but had no effect if added during the cytotoxicity test. It is suggested that specific antigenic triggering may have caused the development of the non-specific killer (effector) cells.

1537 EFFECT OF ENDOGENOUS AND EXOGENOUS MURINE LEUKEMIA VIRUS INFECTION ON IMMUNOLOGIC RESPONSE. (Eng.) Collavo, D. (Inst. Pathological Anatomy, Univ. Padova, Padova, Italy); Biasi, G.; Colombatti, A.; Chieco-Bianchi, L. *Eur. J. Cancer* 11(6):443-449; 1975.

Thymus derived (T) and bursa equivalent derived (B) lymphocyte functions of AKR and C58 mice, natural carriers of endogenous Gross-murine leukemia virus

(MuLV), were compared to those of normal passage A Gross- and Graffi-MuLV neonatally injected CBA mice. The same percentage of θ positive T lymphocytes was found in blood, spleen and lymph node from normal donors of the three strains. Results of phytohemagglutinin (PHA) stimulation of spleen cells *in vitro* disclosed depressed values for the leukemia-prone strains. Moreover, only C58 spleen cell cultures stimulated by lipopolysaccharide (LPS) were depressed. However, no significant reduction in PHA and LPS *in vitro* response was observed in spleen cultures from passage A Gross and Graffi-MuLV neonatally injected, leukemia-free CBA mice. Following *in vivo* immunization with Sheep RBC, AKR mice produced significantly less plaque forming cells (PFC) than both C58 and CBA mice. After immunization with LPS, both AKR and C58 mice produced significantly less PFC than CBA mice. In leukemia-free, passage A Gross- and Graffi-MuLV neonatally injected CBA mice, similarly immunized with Sheep RBC and LPS, a slight but significant reduction in the PFC number against Sheep RBC was found only in Graffi-MuLV-injected mice. The remaining groups gave values similar to their controls. It is concluded that in comparison to CBA mice, an impairment of T and/or B lymphocyte functions exists in leukemia-prone AKR and C58 mice.

1538 PRIMARY AND SECONDARY *IN VITRO* GENERATION OF CYTOLYTIC T LYMPHOCYTES IN THE MURINE SARCOMA VIRUS SYSTEM. (Eng.) Plata, F. (Ludwig Inst. Cancer Res., Lausanne, Switzerland); Cerottini, J. C.; Brunner, K. T. *Eur. J. Immunol.* 5(4):227-233; 1975.

Cell-mediated cytotoxic responses *in vitro* to surface antigens associated with murine sarcoma virus (MSV)-induced tumors were investigated using mixed leukocyte-tumor cell cultures (MLTC). The source of responding cells was either spleens from normal C57BL/6 mice (primary MLTC) or spleens of C57BL/6 mice carrying or having rejected a MSV-induced tumor (secondary MLTC). Graffi virus-induced GiL-4 leukemia cells, Rauscher virus-induced RB1-5 leukemia cells, and MSV-induced MSV-B16 sarcoma cells were used as stimulating syngeneic tumor cells and/or target cells. Cytolytic T lymphocytes (CTL) were generated in both primary and secondary MLTC. A quantitative short-term ^{51}Cr release assay system showed CTL activity in secondary MLTC populations was at least 10-fold higher than that in primary MLTC populations, and 100-fold higher than that in spleen cells taken at the peak of the response of infected mice. The ability of spleen cells to mount a secondary CTL response *in vitro* could be observed as early as five days after virus injection, increased up to the time of maximum tumor size and persisted long after tumor regression. This suggests the development of increased numbers of CTL progenitors and/or formation of "memory" CTL in spleens of infected mice. The results indicate that under appropriate culture conditions, CTL directed against MSV-associated surface antigens can be generated in MLTC.

1539 EPSTEIN-BARR VIRUS BINDING SITES ON LYMPHOCYTE SUBPOPULATIONS AND THE ORIGIN OF LYMPHOBLASTS IN CULTURED LYMPHOID CELL LINES AND IN THE BLOOD OF PATIENTS WITH INFECTIOUS MONONUCLEOSIS. (Eng.) Greaves, M. F. (Dep. Zool., Univ. Coll., London, England); Brown, G.; Rickinson, A. B. *Clin. Immunol. Immunopathol.* 3(4):514-524; 1975.

A fluorescent antibody technique was used to demonstrate the binding of Epstein-Barr virus (EBV) to lymphocytes obtained from tonsil, thymus, fetal spleen and blood from healthy and infectious mononucleosis (IM) patients. Extracts of 16 different virus-producing lymphoid cell lines (LCL) were added to lymphocyte suspensions followed by the addition of fluoresceinated EBV antibody. Fetal spleen, tonsil, blood and thymus lymphocytes demonstrated EBV-binding in 71, 44, 16, and <0.5% of the cells, resp. Combined EBV-binding and T and B cell surface marker tests showed that the binding of EBV occurs on B lymphocytes. Lymphocyte preparations from children with selective immunodeficiencies and purified T and B cell preparations gave similar results. EBV-binding was demonstrated by 90% of the cells of an IgG-secreting myeloma, but did not occur in acute lymphoblastic leukemia, chronic lymphocytic leukemia or T cell-type chronic lymphocytic leukemia cells. Cell surface marker analysis of the 16 LCL revealed that 15 of them were B cell-like and only one was T cell-like. Cell surface marker analysis of atypical IM cells revealed them to be predominantly T lymphoblasts, although B lymphocytes were also present. The results suggest that cells of B lymphocyte origin are the cells capable of latent virus infection *in vivo*, and that these cells may be similar to the atypical B cells seen in IM patients.

1540 BIOLOGIC AND ANTIGENIC SIMILARITY OF VIRUS-INDUCED MIGRATION INHIBITION FACTOR TO CONVENTIONAL, LYMPHOCYTE-DERIVED MIGRATION INHIBITION FACTOR. (Eng.) Yoshida, T. (Univ. Connecticut Health Cent., Farmington); Bigazzi, P.; Cohen, S. *Proc. Natl. Acad. Sci. USA* 72(4):1641-1644; 1975.

A macrophage migration inhibition factor (MIF_V) obtained from supernatants of SV40-infected African green monkey kidney cell cultures was compared with a conventional factor (MIF_L) obtained from supernatants of cultures of lymphocytes taken from Hartley albino guinea pigs which had been immunized by foot pad injections with dinitrophenylated bovine serum albumin. The preparations were subjected to Sephadex G-100 gel fractionation before use in assays and other tests. When administered i.v. to guinea pigs immunized with diphtheria toxoid-antitoxin complex, MIF_V caused a 79.2% reduction in skin reactivity and MIF_L caused, similarly, an 82.9% reduction. Adsorption with columns of anti-MIF_L lymphokine bound to agarose beads removed virtually all activity from both MIF preparations, demonstrating antigenic similarity. Hence, it was suggested that MIF_V and MIF_L may be identical molecular species.

1541 CONCANAVALIN A RECEPTORS IN THE PLASMA MEMBRANE OF RAT LIVER CELLS: COMPARATIVE ELECTRON MICROSCOPIC STUDIES ON NORMAL CELLS AND ON CELLS *IN VIVO* TRANSFORMED BY DIETHYLNITROSAMINE. (Eng.) Roth, J. (Pathologisches Institut der Friedrich-Schiller-Universitat, DDR - 69 Jena, Ziegelmuh-

lenweg 1, East Germany); Neupert, G.; Bolck, F. *Exp. Pathol. (Jena)* 10(3/4):143-155; 1975.

The Concanavalin A (Con A) receptors at the cell surface of normal rat liver cells and of those transformed *in vivo* by diethylnitrosamine were compared by electron microscopic cytochemistry. Metastasizing hepatocellular carcinomas were induced *in vivo* in 20 male Wistar rats by administration of 0.0075% diethylnitrosamine in drinking water. The entire liver was resected, and the carcinomatous tissue regions were excised for cell culture. Contact-inhibited diploid rat liver cells (line RL 19) were used as controls. The Con A-peroxidase reaction, which was used for the electron microscopic visualization of particular terminal sugar moieties of cell surface carbohydrates, was carried out *in situ* within the culture flasks at 37 C. Morphometrical analyses were carried out on micrographs taken at 10,000 X and enlarged three times photographically. The cells were incubated with Con A (50, 100, 250, and 500 μg/ml for 30 min at 37 C and studied by light microscopy. At concentrations between 50 μg/ml and 250 μg/ml, the normal rat liver cells were not agglutinated. Moderate agglutination was observed only at 500 μg/ml. The transformed liver cells were agglutinable by Con A in all concentrations. In the plasma membrane of normal liver cells, a continuous cell surface stain, rather uniform in thickness, was observed after the Con A-peroxidase reaction; a patchily distributed reaction product was visible at the plasma membrane of transformed cells. In the transformed cells, alteration of the surface profile was obvious. In addition to abundant microvilli, invaginations of the plasma membrane were observed. The microvilli generally remained unstained, whereas membrane invaginations as well as pinocytotic vesicles showed a positive reaction. Analyses by quantitative morphometry indicated that in transformed liver cells the portion of labeled cell surface areas was lower (81-60.2% of the cell surface), than in normal liver cells (97-87.5% of the cell surface). The results may indicate an enhanced mobility of the lectin receptors and a lowered stability of the plasma membrane after malignant transformation.

1542 DISTRIBUTION OF CONCANAVALIN A BINDING SITES ON THE SURFACE OF DISSOCIATED RAT SUBMANDIBULAR GLAND ACINAR CELLS. (Eng.) Amakawa, T. (Tokyo Metropolitan Inst. of Gerontology, 35-2 Sakaecho, Itabashiku, Tokyo-173, Japan); Barka, T. *J. Histochem. Cytochem.* 23(8):607-617; 1975.

The submandibular glands of four-wk old rats were dissociated by a procedure involving digestions with collagenase and hyaluronidase, chelation of divalent cations and mechanical force. A suspension of single cells was obtained in low yield by centrifugation 60 x *g* in a Ficoll-containing medium. Immediately after dissociation and after a culture period of 16-18 hr, the dissociated cells were tested for agglutinability by concanavalin A (Con A). Using ferritin (Fer)-conjugated Con A, the lectin binding by the isolated acinar cells was also studied. The dissociated cells were agglutinated by low concentrations of Con A and bound Fer-Con A molecules on their entire surface without any indication of polarization of the cell membrane. There was a considerable cell to cell variation in the amount of Fer-Con A binding which was, in general, sparse and patchy. The contact surfaces between agglutinated cells revealed a dense binding of Fer-Con A molecules irrespective of the types of cells participating in the agglutination reaction. Cells cultured for 16-18 hr were no longer agglutinated by Con A. As compared to the freshly dissociated cells, the cultured acinar cells revealed a more uniform and denser binding of Fer-Con A molecules. Furthermore, there were more lectin molecules bound to the cell surface corresponding to the basal part of the cell, where the nucleus and most of the rough surface endoplasmic reticulum were located, than to the apical cell surface. It is suggested that the higher density of lectin-binding sites on the cell surface in the vicinity of the cisternae of the rough endoplasmic reticulum indicates insertion sites of newly synthesized membrane glycoproteins.

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1598 FRIEND LEUKEMIA VIRUS-INDUCED SUPPRESSION OF LYMPHOCYTE MEDIATED CYTOTOXICITY [abstract]. (Eng.) Mascio, A. A. (Pennsylvania State Univ., University Park); Ceglowski, W. S. *Fed. Proc.* 34(3):973; 1975.

See also:

* (Rev): 1213, 1214, 1239, 1240, 1241, 1242, 1243, 1244
* (Chem): 1268
* (Viral): 1398, 1465, 1473, 1476, 1478
* (Path): 1600, 1601, 1613, 1653, 1698

1599 THE CELLULAR REACTION DURING CARCINOGENESIS: VARIATIONS IN THE NUMBERS OF MAST CELLS, PLASMA CELLS AND EOSINOPHILS. (Eng.) Healy, T. M. (Dept. Pathology, Univ. Coll., Dublin, Ireland). *Ir. J. Med. Sci.* 144(7):255-265; 1975.

Squamous carcinoma was induced in Syrian hamsters in order to determine if there was any correlation between the epithelial changes at the various stages of neoplasia and the changes in number and type of inflammatory cells in the underlying stroma. The cheek pouches were painted with 2% 9:10 dimethyl-1:2 benzanthracene three times weekly for 13 wk, after which tumors developed and the animals were killed. The underlying cellular reaction of lymphocytes, plasma cells, mast cells and eosinophils was studied quantitatively during the various stages of development of the tumors. Between the early stage of hyperplasia and that of squamous carcinoma, the eosinophils increased from 2% to 17% of all the cells present under the growths. There was a complementary fall in plasma cells as malignancy developed, but the lymphocytes and mast cells remained at a fairly constant level. Possible explanations for these changes include a direct reaction to the chemical carcinogen, or a nonspecific inflammatory response by the host to the presence of abnormal epithelium.

1600 MULTIPLE PRIMARY MALIGNANT NEOPLASMS. A SEARCH FOR AN IMMUNOGENETIC BASIS. (Eng.) Dellon, A. L. (Natl. Cancer Inst., Bethesda, Md.); Chretien, P. B.; Potvin, C.; Rogentine, G. N. Jr. *Arch. Surg.* 110(2):156-160; 1975.

To determine if the occurrence of multiple primary malignant neoplasms has an immunogenetic basis, cellular immunity studies and human leukocyte antigen (HL-A) typing were conducted on 42 patients with 2-4 primary malignant neoplasms. Nine patients were studied for an average of 1.9 yr preceding radiation therapy. A microcytotoxicity test was used for HL-A typing, and thymus-dependent lymphocytes (T cells) were determined by rosette formation of sheep RBC with patient's peripheral blood lymphocytes. The T cell levels were significantly depressed only among patients who had received radiation therapy or who carried metastatic tumors. Abnormal frequencies of certain HL-A antigens were not exhibited. Thus, depressed T cell levels are not a probable basis for the development of multiple primary malignant neoplasms, and abnormal distribution of HL-A antigen frequencies also are not indicative of the condition. Multiple primary malignant neoplasms often occur in related tissues, e.g. lung/larynx/oral cavity, and they also occur successively at a time interval consistent with the patient's being cured of preceding malignant neoplasms. This suggests that the presumed "increased susceptibility to cancer" is actually a result of repetitive induction by the same or similar etiologic factors in patients who are cured after treatment of the first malignant neoplasm.

1601 IgD MULTIPLE MYELOMA: REVIEW OF 133 CASES. (Eng.) Jancelewicz, Z. (Wellesley Hosp., Toronto, Canada); Takatsuki, K.; Sugai, S.; Pruzanski, W. *Arch. Intern. Med.* 135(1):87-93; 1975.

Data obtained from the histories of 133 patients with IgD myeloma are reviewed in comparing this myeloma with myelomas having other M-components. Sera and concentrated urines were tested by electrophoresis and by immunoelectrophoresis, employing anti-sera against IgG, IgA, IgM, IgD, IgE and Bence Jones κ-type and λ-type proteins. Immunoquantitation of all immunoglobulins except IgE was performed. Mean age at diagnosis of the myeloma was 56.2 years with males predominating. Fifty-five percent of 65 examined patients had lymphadenopathy, hepatomegaly, or splenomegaly or a combination of these. Osteolytic lesions were seen in 79% of 120 patients examined, azotemia in 67% of 21 patients, and severe anemia (hemoglobin less than 10 g/100 ml) in 61% of 84. Total serum protein level was less than 8.0 g/100 ml in 71% of 87 patients. IgD M-component levels were often low. λ-type light chains were found in 90% of IgD M-components. Bence Jones proteinemia occurred in 71% of 41 patients and Bence Jones proteinuria occurred in 92% of 119. Mean survival was 13.7 months from time of diagnosis. The IgD M-components comprised 0.8% of M-components in general and 2.1% of myelomas in particular. Comparison of patients with IgD κ-type M-component and those with λ-type light chains showed no clinical difference. IgD myeloma is different from IgG and IgA myelomas, indicating that the clinical picture and course of multiple myeloma may be related to the class and type of M-component.

1602 LIGHT AND ELECTRON MICROSCOPIC STUDIES OF THE BONE MARROW AND BLOOD CELLS IN CHRONIC PANMYELOSIS INCLUDING POLYCYTHEMIA VERA AND PRIMARY THROMBOCYTHEMIA. (Eng.) Nagai, K. (Hirosaki Univ. Sch. Medicine, Hirosaki, Japan); Kamata, Y.; Kimura, M.; Chiba, Y. *Acta Pathol. Jpn.* 25(5):517-537; 1975.

Bone marrow and peripheral blood of patients with polycythemia vera (three cases), primary thrombocythemia (two cases), and panmyelosis (one case) were examined with the light and electron microscopes. In five cases the peripheral blood showed persistent increase in cells of 2 or 3 hematopoietic systems. Giant thrombocytes in the peripheral blood were seen in three cases. Erythroblasts, granulocytic young forms, and megakaryocytes were often observed in the blood. Histologic bone marrow examination showed prominent proliferation of all three hematopoietic cells in every case. Cytological and electron microscopic examinations of the bone marrow revealed many mitotic figures, morphological abnormalities, and unbalanced nucleocytoplasmic maturation in various hematopoietic cells. These findings suggest that the proliferation of all three hematopoietic cells in the bone marrow was not simply reactive in nature, but rather an idiopathic progressive process. The authors suggest that these disorders represent no separate entities and must be unified as "chronic panmyelosis".

1603 ON THE BIOLOGICAL BEHAVIOUR OF BASALIOMAS. (Eng.) Koch, H. (Clinic for Maxillo-Facial and Plastic Surgery of Face, Westdeutsche Kieferklinik, Univ. Düsseldorf, W. Germany); Pfeifle, K.; Kreidler, J. *Maxillofac. Surg.* 3(1):35-36; 1975.

Arguments against the malignancy of basaliomas are

presented. Based on experience in 250 cases, the cords of basalioma grow infiltratively. They have no basal membrane, are not defined by a capsule, and are incorporated in a fibrous tumor stroma. This form of growth is no proof of malignancy since a similar form is shown by hemangioma or neurofibroma. Random multiplicity was observed in only about 10% of patients with basaliomas. Recurrence of the sclerodermiform basalioma as well as the cicatrising type is the result of improper treatment. Based on U. S. morbidity statistics, only 0.05% of basaliomas metastasize; this does not justify the classification of basalioma as generally malignant. Although forms transitional to carcinoma are shown by basaliomas, no mixed form has developed from progressive degeneration of tumor cells passing through an intermediate metasplastic form of basalioma.

1604 ADENOID CYSTIC CARCINOMA OF THE BREAST: PREVALENCE, DIAGNOSTIC CRITERIA, AND HISTOGENESIS. (Eng.) Anthony, P. P. (Middlesex Hosp. Med. Sch., London W1, England); James, P. D. *J. Clin. Pathol.* 28(8):647-655; 1975.

Three cases of adenoid cystic carcinoma were identified in a ten-year review of 2686 cases of breast carcinoma. The criteria necessary for diagnosis are reviewed with particular reference to cribriform intraduct carcinoma and adenocarcinoma of the breast with a small, dark, 'basaloid'-cell pattern. The most important single diagnostic criterion of adenoid cystic carcinoma is a biphasic cellular pattern which may be aided by the demonstration of two types of mucin-stromal acid mucopolysaccharide and ductal neutral mucopolysaccharide. This tumor most frequently presents as a painful or tender mass near the areola, and it carries a uniquely favorable prognosis when compared with similar tumors elsewhere in the body. Actomyosin was demonstrated in all three tumors by an immunofluorescent method, and this supports a predominantly myoepithelial origin.

1605 APOCRINE METAPLASIA IN CYSTIC HYPERPLASTIC MASTOPATHY: HISTOCHEMICAL AND ULTRASTRUCTURAL OBSERVATIONS. (Eng.) Ahmed, A. (Dept. of Pathology, Univ. of Manchester, England). *J. Pathol.* 115(4):211-214; 1975.

The histochemical and ultrastructural features of apocrine metaplasia were described during an investigation of 53 cases of cystic hyperplastic mastopathy of the human breast. Acid phosphatase showed a strong reaction along the luminal border of the epithelium. The alkaline phosphatase reaction was confined to the myoepithelial cells and adjacent blood vessels. Apocrine-type epithelium showed strong succinic and LDH activity and an intense NADH diaphorase reaction. Electron microscopy revealed that the cells resembled the epithelium of apocrine sweat glands: pale, large, columnar, and eosinophilic cells. The cytoplasm was rich in organelles with an increase in the number of mitochondria. Numerous infoldings of the basal plasma membrane were evident. These observations support the view that this lesion is a metaplastic change in normal breast epithelium, and not the result of degeneration.

1606 TWO PSEUDODIPLOID HUMAN BREAST CARCINOMAS STUDIED WITH G-BAND TECHNIQUE. (Eng.) Mark, J. (Central Hosp., Skovde, Sweden). *Eur. J. Cancer* 11(11):815-819; 1975.

A cytogenetic study on two human breast carcinomas was carried out using a G-band technique. The poorly differentiated scirrhous neoplasms were removed from a 78-yr-old woman and from a 74-yr-old woman. In the first case, 27 cells were karyotyped; in the second case, 44 cells were karyotyped. Both tumors had a pseudodiploid stemline. The first case had the constitution 46,XX,2p-,+5,+8,9p-,16q-,17p+,-20, -21; the details of the structural rearrangement could be completely clarified. The other stemline had the constitution 46,XX,3q-,-6,+7,-8,11q+,-15, 16q+,+2 different ring chromosomes; the details of the structural changes could be clarified to a considerable extent. A preferential pattern of chromosome variation in breast carcinoma is discussed on the basis of the present results and a review of the literature.

1607 G- AND C-BANDING PATTERNS IN THE T2 MURINE LEUKEMIA. (Eng.) Bianchi, M. S. (Instituto Multidisciplinario de Biologia Celular, Calle 526 entre 10 y 11, La Plata, Argentina); Merani, S.; Bianchi, N. O. *J. Natl. Cancer Inst.* 55(4):1017-1021; 1975.

The bone marrow cells of BALB/c mice with T2 murine leukemia were analyzed cytogenetically. Three female and four male mice with T2 viral leukemia in passages 69 and 96 were given injections of 1 μg colchicine/g; three hours later they were killed, and chromosome spreads were prepared by the air-drying methods. Of 98 leukemia metaphases, 16.4% were hypodiploid, 4.1% hyperdiploid, and 79.5% diploid. The distribution of G bands in diploid metaphases indicated that almost half of them were pseudodiploid, with chromosome abnormalities such as trisomies, monosomies, nullisomies, unidentified chromosomes, translocations, deletions, or duplications. Since all mouse chromosomes are acrocentric and can be identified only tentatively, most of the anomalies detected with G-banding procedures would have passed unnoticed with conventional cytogenetic techniques. The C-banding pattern of leukemia cells did not differ from that of normal controls. However, a considerable number of leukemia cell metaphases had bridges connecting the centromeric C bands of two or more chromosomes. This phenomenon probably indicates an increased stickiness of the heterochromatin, which may produce mitotic nondisjunction and the appearance of monosomies and trisomies.

1608 TRISOMY OF CHROMOSOME 15 IN SPONTANEOUS LEUKEMIA OF AKR MICE. (Eng.) Dofuku, R. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Biedler, J. L.; Spengler, B. A.; Old, L. J. *Proc. Natl. Acad. Sci. U.S.A.* 72(4):1515-1517; 1975.

Karyotype analysis by trypsin-Giemsa banding was conducted on spontaneous thymomas in AKR/J female mice. An initial survey using a conventional staining technique to determine chromosome number indicated that at least 1/3 of the mice with thymomas would be apt to yield cells with 41 chromosomes. Most cells in this study did have 41 chromosomes. Of the 11 thymomas studied, 10 exhibited trisomy 15 in some portion of the cells. Seven tumors were predominately trisomic for chromosome 15. One thymoma was trisomic for chromosome 12, while another exhibited multiple trisomy for chromosomes 3, 12, 15, and 17. All cells lacked one chromosome, but two cells were trisomic for chromosome 15 in a mouse with a modal number of 39. A thymoma with a modal number of 40 had one cell exhibiting trisomy 15 and another cell with trisomy 10. No evidence of trisomy was detected in 13 normal AKR mouse thymuses or in mouse embryos. Chromosome 15 is frequently present in a trisomic state in spontaneous lymphomas of AKR mice. The author concludes that: (1) reproducible and specific chromosomal changes are associated with tumor development, (2) different etiologic agents induce different chromosomal changes, and (3) trisomy of specific chromosomes is the most frequent and consistent concomitant of these karyotypic changes.

1609 CHROMOSOME ABNORMALITIES IDENTIFIED BY BANDING TECHNIQUE IN A PATIENT WITH ACUTE MYELOID LEUKAEMIA COMPLICATING HODGKIN'S DISEASE. (Eng.) Lundh, B. (Department of Internal Medicine, Lasarettet, S-22185 Lund, Sweden); Mitelman, F.; Nillsson, P. G.; Stenstam, M.; Söderström, N. *Scand. J. Haematol.* 14(4):303-307; 1975.

Chromosome analysis using trypsin-Giemsa banding technique was performed on the bone marrow cells of a 38-yr-old man with acute myeloid leukemia and Hodgkin's disease in order to compare the two malignancies. Fifty cells were counted and 19 cells were karyotyped in detail. Of the 50, 12 showed 46 chromosomes; 30 had 47 chromosomes; six had 48 chromosomes; and two had 49 chromosomes. All cells examined with 47 chromosomes had the abnormal karyotype of an extra chromosome 14, loss of one chromosome 17, and gain of one chromosome 18. These changes were also present in eight cells analyzed with 46 and 48 chromosomes. These abnormalities, similar to the karyotypic changes of lymphoid cells in malignant lymphomas, suggest a pathogenic and/or etiologic relation between the two disorders.

1610 ISOCHROMOSOME 17 IN PROSTATIC CANCER. (Eng.) Oshimura, M. (Roswell Park Memorial Inst., Buffalo, N.Y.); Sandberg, A. A. *J. Urol.* 114(2):249-250; 1975.

A chromosomal picture of a metastatic cancer of the prostate is described and the first description of an isochromosome 17 marker in a human cancer is related. Direct bone marrow chromosome preparations showed a mode of 70 chromosomes but with considerable scatter in counts around this mode and the presence (about 15%) of normal diploid metaphases with 46 chromosomes, undoubtedly of normal bone marrow origin. Staining with Q and G banding techniques was used to examine chromosome structures on eight abnormal metaphases. This procedure revealed remarkable numerical and structural karyotypic changes, including the presence of two ring chromosomes and a missing Y chromosome in every metaphase scrutinized. The new banding techniques have revealed the presence of a marker thought to be an isochromosome of the long arm of 17 in several cases of chronic myelocytic leukemia, in a case of acute myelocytic leukemia and in a case of acute lymphocytic leukemia. Further precise identification of individual chromosomes in other malignant diseases may reveal the occurrence of isochromosome 17 to be a frequent phenomenon in human cancer.

1611 CHROMOSOME AND HISTOLOGIC PATTERNS IN PRE-INVASIVE LESIONS OF THE CERVIX. (Eng.) Stanley, M. A. (Queen Elizabeth Hosp., Woodville, Australia); Kirkland, J. A. *Acta Cytol.* 19(2):142-147; 1975.

The chromosome constitution and histologic appearance of seven cases of preinvasive carcinoma of the cervix are reported. All patients were subjected to colposcopy and subsequent biopsies as a result of a series of abnormal cytologic smears. Punch biopsies using colposcopic localization, taken from the area of maximal abnormality, and unfixed, freshly excised cone biopsies were examined at two different time intervals. Squash preparations, examined with phase microscopy, were used in chromosome analysis. The cytologic history of all patients indicated a lesion bordering on severe dysplasia or carcinoma *in situ*; in four, the histologic diagnosis was the same in both biopsies, while three progressed from dysplasia to carcinoma *in situ*. The results of chromosome analysis revealed that the chromosome profile remained stable in six cases, with no shift in chromosome complement occurring and the majority of cells exhibiting pseudodiploid arrangements. However, a shift in chromosome complement did occur in one case, as reflected in the histology of both biopsies. Thus, the histologic pattern and chromosome profile remained constant during the period between biopsies (an average of five months). This case illustrates the importance of biopsy techniques in chromosome studies in preinvasive lesions; the correlation of histologic pattern and chromosome complement is only possible when the biopsy can be localized precisely. The very wide range in chromosome number in carcinoma *in situ* might be regarded as a situation in which various new combinations of chromosomes constantly arise; cell surfaces thus altered are being tested in the epithelium of the individual. In such a situation invasion will occur when a cell with the appropriate chromosome complement and antigenic status arises.

1612 SOMATIC REARRANGEMENT OF CHROMOSOME 14 IN HUMAN LYMPHOCYTES. (Eng.) McCaw, B. K. (Univ. Oregon Health Sciences Center, Portland, Oreg. 97201); Hecht, F.; Harnden, D. G.; Teplitz, R. L. *Proc. Natl. Acad. Sci. USA* 72(6):2071-2075; 1975.

The lymphocytes from eight patients with ataxia-

langiectasia (A-T) with progressive cerebellar axia, oculocutaneous telangiectases, immunodeficncy, and pedigrees consistent with autosomal ressive inheritance were karyotyped by Q-banding acetic-saline-Giemsa method for G-banding in der to relate chromosomal rearrangement with lymphd malignancy development. All clones were marked a structural rearrangement of the long arm (q) chromosome 14. This rearrangement was a cleart translocation with two exceptions: an elongated romosome 14 in one case, and a ring 14 in another se. The breakpoints were clustered around the 2 band of the chromosome. Loss or gain of chromomal material was not detectable by measurement. her chromosomes which participated in the transcation process were 14 homolog, 6, 7, and the X. e proportion of lymphocytes containing the reranged chromosome 14 varied with each patient; ually the percentage increased with time. The ttern of chromosomal change was nonrandom, sugsting that a 14q rearrangement is the initial romosomal change in lymphocyte clone of A-T paents. The structural rearrangement of chromosome may be directly related to abnormal growth of mphocytes and may be a step toward the developnt of lymphoid malignancies.

13 A HUMAN/MOUSE HYBRID MODEL FOR THE STUDY OF HUMAN GENETIC FACTORS INFLUENCING TUMOR LL GROWTH. (Eng.) Koshman, R. W. (Dept. Surgery d Surgical-Medical Res. Inst., Univ. Alberta, Ednton, Alberta, Canada); Koo, J.; Thurston, O. G. *Surg. Oncol.* 7(4):323-327; 1975.

matic cell hybridization (fusion) was carried out tween cells of the murine lymphoma L-5178Y(r) and ripheral human lymphocytes. The L-5178Y(r) line deficient in hypoxanthine-guanine phosphorybosyl ansferase (HGPRT). Using a selective medium sysm (hypoxanthine, methotrexate, and thymidine), it s possible to isolate hybrid clones having the mplete complement of murine chromosomes as well as e human X chromosome on which the gene for HGPRT located. This model system can be used to study e effect of the human X chromosome on the phenope of the murine lymphoma *in vitro*. Since the 5178Y(r) lymphoma can be grown as a solid or ascic tumor in C3H and many other mouse strains, it y also be possible to study the effect of X-linked nes on the behavior of the tumor *in vivo*.

14 THE LYMPHATIC DISSEMINATION IN ENDOMETRIAL CARCINOMA: A STUDY OF 188 NECROPSIES. ng.) Henriksen, E. (1136 W. Sixth St., Los Angeles, lif. 90017). *Obstet. Gynecol.* 123(6):570-576; 75.

more clearly define the biologic activity of endotrial carcinoma, 188 cases selected at random were udied at necropsy. In 51% of the cases death was tributed to the endometrial carcinoma. Disseminaon was more scattered and less predictable than s been reported in cervical cancer; the biological tential was not invariably based on the site of e primary lesion, the depth of invasion, or the stologic differentiation. Difficulties in performing a thorough examination of the lymphatic system in this series included the increased age of the patients and a higher incidence of medical diseases and surgical procedures. The data illustrate the erratic natural history of the disease, but do not permit the factual incidence of node or organ involvement.

1615 THE NATURAL HISTORY OF A GIANT-CELL TUMOR: CASE REPORT. (Eng.) Vistnes, L. M. (Veterans Admin. Hosp., 3801 Miranda Ave., Palo Alto, Calif. 94304); Vermuelen, W. J. *J. Bone Joint Surg.* [*Am.*] 57A(6):865-867; 1975.

The development of a giant cell tumor, which occurred in a 48-yr-old Samoan woman, could be documented for more than two years before treatment. The tumor occurred in the proximal phalanx of the left finger. The initial roentgenogram showed an osteolytic lesion occupying both epiphyseal and metaphyseal areas; the metacarpophalangeal joint was intact, as was the bone cortex, except for an area of break-through adjacent to the metacarpophalangeal joint of the long finger. The patient had been treated with herbal preparations for 27 mo. At the time of readmission, the tumor was 7 cm in diameter and its ulcerated surface involved an area 5 cm in diameter. A preoperative roentgenogram showed complete loss of recognizable architecture of the proximal phalanx of the index finger, with destruction of the metacarpophalangeal joint and spread of tumor locally to involve the distal index metacarpal and cause erosion of the radial portion of the adjacent metacarpophalangeal joint. When the tumor was removed, it was clear that the erosion represented direct spread of the tumor in that the entire capsule of the joint was necrotic; the tumor involved both the joint and the flexor tendon mechanism in the area. The long finger was therefore amputated in continuity with its distal metacarpal. Adequate wound closure was obtained with the filleted flap technique. Histologic examination of the resected tumor showed no evidence of malignancy, and there was no sign of recurrence one year after surgery. Treatment of this type of hand tumor given at an earlier stage than in the present case could consist of total excision of all the bone involved and immediate bone grafting to preserve the function of the digit.

1616 GRANULAR CELL TUMOR (MYOBLASTOMA) OF THE ORBIT. (Eng.) González-Almaraz, G. (Gen. Hosp. Mexico City, Mexico); de Buen, S.; Tsutsumi, V. *Am. J. Ophthalmol.* 79(4):606-612; 1975.

A case of granular cell myoblastoma of the orbit was studied histochemically and by light, phase contrast, and electron microscopy. An 8-yr-old Caucasian boy presented initial clinical manifestations of conjunctival hyperemia, progressive left exophthalmos with epiphora, headache, and reduced visual acuity, leading to a clinical diagnosis of muscle cone syndrome. A biopsy of the subconjunctival tumor on the nasal side of the left eye was performed, resulting in the histopathologic diagnosis of granular cell myoblastoma; the whole tumor

and eyeball were exenterated. The tumor was brown, ovoid in shape, measured 35 x 25 x 24 mm, and well encapsulated with a nodular and vascularized surface. It was composed of compact irregular strands and lobules of large polyhedral and round cells, with round nuclei and finely coarse chromatin. Phase microscopy, employing a variety of staining techniques, demonstrated two types of cells, both showing birefringent round and rod-like inclusions of varied sizes. Electron microscopy showed cells in different stages of physiologic activity; cells with a clear cytoplasmic matrix, rich in mitochondria and conspicuous granular endoplasmic reticulum were most frequent. Other cell types included those with an intermediate dense cytoplasmic matrix, plus occasional cells of a dense matrix. The granular cell myoblastoma is considered to be a benign neoplasm, with the three types of cells described representing different stages of maturity or differentiation and metabolic function. The results support the hypothesis that the granular cells are of histiocytic origin; "granular cell histiocytoma" may more accurately describe this neoplasm.

1617 ULTRASTRUCTURE OF SPONTANEOUS AND URETHAN-INDUCED THYMOMAS IN BUFFALO RATS. (Eng.) Matsuyama, M. (Aichi Cancer Center Res. Inst., Chikusa-ku, Nagoya, Japan); Suzuki, H.; Yamada, S.; Ito, M.; Nagayo, T. *Cancer Res.* 35(10):2771-2779; 1975.

Normal thymuses from Buffalo and Long-Evans rats of various ages (two days, one month, and six months for Buffalo rats, and 12 and 18 mo for Long Evans rats), and spontaneous and urethan-induced thymomas in Buffalo rats, were examined by electron microscopy. Histological variabilities among thymomas of the lymphoid, mixed, and epithelial cell types were a reflection of the number of lymphoid cells within the network composed of neoplastic epithelial reticular cells. In the cytoplasm of these cells, development of tonofilaments and membrane-bound bodies and inverse development of the rough-surfaced endoplasmic reticulum were recognized in the sequential process from the lymphoid cell type to the epithelial cell type. Thus, an important role of the development of the rough-surfaced endoplasmic reticulum for thymic function is suggested. Phagocytic activity of the neoplastic epithelial reticular cells was revealed, and some of the membrane-bound bodies in these cells, especially those with moniliform structures, were regarded as remnants of damaged lymphocytes. Evidence for neoplastic epithelial reticular cell-lymphoid cell transformation could not be established from study of the thymoma tissue. No virus-like structures were observed in these thymomas.

1618 CHANGES IN ULTRASTRUCTURE OF RAT OVARIES AFTER EARLY POSTNATAL X-RAY IRRADIATION. (Eng.) Matsumoto, A. (Juntendo Univ. Sch. Med., Tokyo, Japan). *Endocrinol. Jpn.* 22(1):1-15; 1975.

Female rats (Wistar/Tw) were irradiated with 190R (40R/min) of X-rays at ten days of age and ovarian ultrastructures were studied by electron microscopy 4 and 7 mos after irradiation. Ultrastructural changes were found in germinal epithelial cells, fibroblasts in the tunica albuginea and interstitial cells. The germinal epithelial cells exhibited signs of degeneration but no sign of proliferation. Electron density of their basal part was reduced considerably. Their mitochondria became swollen and free ribosomes decreased in number. The nuclei often protruded from the free surface of these cells. These cells frequently fragmented and, finally, complete desquamation occurred. The basement membrane became unevenly thickened. Nuclei of enlarged fibroblasts in the tunica albuginea became irregularly ellipsoidal in shape, and the nuclear envelope was occasionally invaginated. Various cytoplasmic organelles of the fibroblasts were well-developed. Some abnormal invasion of cytoplasm into the nucleus was found in the interstitial cells showing the ultrastructural characteristics of steroid hormone synthesis. Various cytoplasmic organelles and inclusions invaded the nucleus of these cells and the nuclear envelope sometimes disappeared locally. The cytoplasmic organelles entering the nucleus were not surrounded by the nuclear envelope. These interstitial cells contained a large number of irregular-shaped electron dense mitochondria with vesicular cristae, and numerous dilated vesicles of smooth-surfaced endoplasmic reticulum. The cells of anovular follicles in the irradiated ovaries resembled, in fine structure, the granulosa cells in normal primary follicles of the non-irradiated ovaries. These cells seem to be less affected by early postnatal irradiation.

1619 FINE STRUCTURE OF DIVIDING CELLS AND OF NON-DIVIDING, DIFFERENTIATING CELLS OF NICKEL SULFIDE-INDUCED RHABDOMYOSARCOMAS. (Eng.) Bruni, C. (Univ. Virginia Sch. Med., Charlottesville); Rust, J. N. *J. Natl. Cancer Inst.* 54(3):687-696; 1975.

Cells of nickel sulfide-induced rhabdomyosarcomas were studied by electron microscopy. Three tumors developed in thigh muscles of rats 155-182 days after the im injection of 20 mg of Ni_3S_2. These cells were compared with normal thigh muscle cells from mature rats, 15-day fetuses and one-day-old rats. Proliferative tumor cells were mononucleate, had many free ribosomes, a few short cisterns of rough endoplasmic reticulum, a few small mitochondria and no myofilaments. They thus exhibited no features of normal muscle cell differentiation. Nonproliferative cells had myofilaments and differentiated similarly to the normal fetus and early postnatal muscle cells; they were similar in myofibril orientation (parallel to the main cell axis) had Z line precursors, cisterns of rough endoplasmic reticulum, many free ribosomes, and the structure and size of mitochondria were similar. Sarcolemma caveolae were larger in the tumor cells than in normal developing cells. In addition, large branching tubules were only present in the tumor. The triads, whose central component is provided by the T system, increased rapidly in the postnatal period but were rare in tumors. These observations indicate defective development of the tumor cell T system. The tumor cells thus did not differentiate beyond the initial stage of triad formation and of the smooth endoplasmic reticulum. Transitional forms between differentiated and undifferentiated tumor cells were

bserved. Only a fraction of the dividing cell pro- eny apparently differentiate; the remaining fraction oes not and remains as proliferating stem cells which ontribute to the growth of the tumor.

520 ULTRASTRUCTURE OF AN ODONTOGENIC MYXOMA. (Eng.) Simes, R. J. (Cátedra de Anatomía atológica, Facultad de Odontología Universidad de uenos Aires, Argentina); Barros, R. E.; Klein-Szanto, . J. P.; Cabrini, R. L. *Oral Surg.* 39(4):640-646; 975.

ne ultrastructural findings in a case of myxoma f the mandible is described. A 43-yr-old woman resented with a localized, painless swelling in ne left side of the lower jaw; odontogenic myxoma as diagnosed by surgical biopsy and routine histo- ogic techniques. Several white, soft, slimy tumor ragments were obtained from a partial resection. ections were stained using hematoxylin and eosin, eriodic acid-Schiff stain, or uranyl acetate and ead citrate. Light microscopy revealed a tumor omposed of numerous stellate and spindle-shaped ells scattered in an abundant, slightly periodic cid-Schiff-positive matrix which contained fibril- ar structures and collagen fibers. By electron icroscopy, the main cells were shown to have ir- egular shape, several intracytoplasmic processes, umerous intracytoplasmic fibrils of 100-150 Å iameter, oval and regular nuclei, moderate clump- g of chromatin, numerous glycogen particles, and olgi complexes. Few mitochondria, free ribosomes, vesicles were present. Some cells exhibited knotic nuclei or other degenerative alterations. ese ultrastructural features were similar to those human cardiac myxoma and the myxoid tissue of arton's jelly. The matrix contained abundant anules averaging 500 Å in diameter and was charac- rized by fine fibrillar projections. The scarce absent ergastoplasm noted in the study could dicate a probable final stage of exhausted secre- ry capability of the cells. The main histologic d ultrastructural features of the myxoma cell e markedly typical, allowing its separate typing om other connective tissue tumors.

21 ULTRASTRUCTURAL CHARACTERISTICS OF HUMAN NEURILEMOMA CELL NUCLEI. (Eng.) Chandra, (Mercy Hosp. and Medical Center, Chicago, Ill. 616); Jerva, M. J.; Clemis, J. D. *Cancer Res.* (8):2000-2006; 1975.

nor tissue and cultured tumor cells from three tients with neurilemoma were examined under the ectron microscope. The nuclei of the neurilemoma lls exhibited deep and extensive invaginations of rt of their surface. These invaginations contained toplasmic matter and exhibited an abundant amount perichromatin material along their membranes. In eas of the nucleoplasm distant from the invagina- ons, neurilemoma cell nuclei contained small mem- ane-bound bodies approximately 130 nm in size. ese bodies, some of which contained a dense "nu- eoid", occurred either singly or grouped together l enclosed within a large membrane body. Although membrane-bound particles have nucleoid and are the same size as herpes-type viruses, they are not considered virus-like particles. Degenerated nuclei in cultured tumor cells contained spherical bodies, 130-230 nm in diameter, with spikes on their surface similar to those seen on envelopes of herpes-type viruses. The significance of nuclear bodies and of small membrane-bound spherical bodies in human neurilemoma tissue is not clear; however, the former has consistently been observed in viral infections both *in vivo* and *in vitro*, concomitant with an increased amount of perichromatin material, as in this study.

1622 BRONCHIOLO-ALVEOLAR CARCINOMA -- CELL OF ORIGIN. (Eng.) Greenberg, S. D. (Baylor Coll. Med., Houston, Tex.); Smith, M. N.; Spjut, H. J. *Am. J. Clin. Pathol.* 63(2):153-167, 1975.

The ultrastructure of five bronchiolo-alveolar carcinomas from three non-smoking women and two non-smoking men was studied to determine the parent cell type. The carcinomas were observed through chest roentgenograms. Electron and light microscope examinations of the tumor tissue revealed two parent cell types, both of brochiolar origin. One cell type was a metaplastic bronchiolar mucous cell while the other was a bronchiolar stem cell that exhibited ultrastructural features of both the respiratory ciliated and respiratory nonciliated cell with little or no mucous secreting ability. Hyperplastic Type II alveolar epithelial cells were found around the margins of these tumors and could be mistaken for the neoplastic cells. Despite the embryologically common origin of the epithelial cells of the bronchiolo-alveolar region and the possibility that tumors of this region may differentiate into cells of the bronchiole or the alveolus, the information accumulated thus far indicates that alveolar-cell carcinoma is a rare occurrence.

1623 ASBESTOS EXPOSURE AND MULTIPLE PRIMARY TUMORS. (Eng.) Dohner, V. A. (Navajo Area Indian Health Service, P.O. Box Drawer "G," Window Rock, Ariz. 86515); Beegle, R. G.; Miller, W. T. *Am. Rev. Respir. Dis.* 112(2):181-199; 1975.

The occurrence of multiple primary tumors in five patients with a history of occupational asbestos exposure is reported. A 47-yr-old man was hospitalized for treatment of pulmonary tuberculosis. He had been exposed to considerable asbestos dust on his job as a shipyard scaler and painter and he had a smoking history of about 40 packs/yr. In December 1966 a moderately well-differentiated squamous cell carcinoma was found in the left lower lobe of the lung, and in August 1969 a squamous cell carcinoma of intermediate differentiation was found in the right lower lobe. A 58-yr-old man was hospitalized in July 1968 with an obstructing napkin-ring adenocarcinoma of the sigmoid colon, and in August 1970 an undifferentiated carcinoma was found in the left lower lobe bronchus. The patient had a 60 pack/yr history of smoking and had had extensive exposure to asbestos dust for over 25 yr as a rigger in the shipyards and a fireman in the Merchant Marine. A 63-yr-old longshoreman with a 45-60 pack/yr history of smoking had handled bags of asbestos

for 20 yr. An adenocarcinoma *in situ* was found near the anal verge in April 1970, and a moderately well-differentiated squamous cell carcinoma was found in the right apex in August 1970. A 66-yr-old patient had a squamous cell carcinoma in the left lower lobe of the lung and an adenocarcinoma in the left upper lobe. He had been a smoker for about 45 yr, and had been exposed to asbestos-containing pipe covering materials for at least 40 yr. Asbestos bodies were frequently seen lying free or within macrophages in the alveoli. In July 1965, a 61-yr-old seaman electrician was found to have a well-differentiated adenocarcinoma within the posterior basal segment of the right lower lobe of the lung. In January 1971, a poorly differentiated carcinoma was seen in the right upper lobe. The patient had had occasional exposure to asbestos between 1946 and 1965 and had a smoking history of 30-35 packs/yr. These cases show that para-occupational exposure to asbestos may occur in workers not involved in the direct use of asbestos; they also show the augmentation effect of cigarette smoking on the risk of asbestos-related lung cancer. The development of lateral pleural thickening and/or diaphragmatic pleural plaques should promote an extensive search for occupational and residential asbestos exposure.

1624 THE "JUVENILE" TYPE OF CHRONIC MYELOGENOUS LEUKEMIA. (Ger.) Terheggen, H. G. (Kinderkrankenhaus der Stadt Köln D-5000 Köln 60 (Riehl) Amsterdamer Strasse 59 Bundesrepublik Deutschland); Haug, H.; Hellriegel, K. P.; Schaefer, H. E. *Z. Kinderheilkd.* 119(2):123-131; 1975.

Juvenile type of chronic myelogenous leukemia in a 8.5-yr-old boy, the first case ever observed at this advanced age, is reported. The juvenile type is characterized by hemorrhagic diatheses, hepatosplenomegaly, generalized enlargement of lymph nodes, reduced erythrocyte and thrombocyte counts, leukemic infiltration of the bone marrow, and decreased activity of leukocyte alkaline phosphatase. Early manifestation of anemia and thrombocytopenia, minor degree of leukocytosis, absence of Philadelphia chromosome, and persistence of fetal hemoglobin and of fetal characteristics of erythrocytic carbonic anhydrases and glucose-6-phosphate dehydrogenase. The occurrence at the age of 8.5 yr suggests the acquired nature of the disorder. The high fetal hemoglobin level in acquired chronic myelogenous leukemia of juvenile type is believed to be due to an acquired disturbance in globin chain synthesis resulting either from the neoplastic cell clone proper or from the depression of beta-chain synthesis with compensatory reactivation of the gamma-chain synthesis.

1625 HAIRY CELL LEUKEMIA ('LEUKEMIC RETICULOENDOTHELIOSIS'), RETICULOSARCOMA, AND MONOCYTIC LEUKEMIA: CYTOCHEMICAL AND ULTRASTRUCTURAL INVESTIGATIONS. (Eng.) Schmalzl, F. (Medizinische Universitätsklinik, A-6020 Innsbruck, Austria); Huhn, D.; Asamer, H.; Braunsteiner, H. *Acta Haematol. (Basel)* 53(5):257-276; 1975.

To determine if any cytological relation exists between monocytic and HC leukemia, the cytochemistry, ultrastructure and functional capacities of leukemic cells from patients with "hairy cell" (HC, 10 patients); monocytic leukemia (17), "reticulosarcoma" (RS, 4), and "reticulosarcoma cell leukemia" (1) were examined. Additional investigations included quantitative determinations of urinary lysozyme excretion, skin window studies (seven patients with monocytic and two with HC leukemia), tests for phagocytosis of ferritin by HC, and labeling of immunoglobulin receptors on HC. Clear evidence against any cytological relation among monocytic and HC-leukemia was provided by ultrastructural, cytochemical, biochemical and immunologic differences and function criteria tests. Further results disagreed with the frequently stressed relation between leukemia monocytes and RS cells. Cases of RS cell leukemia were reclassified as lymphosarcoma cell leukemia, acute lymphatic, and myeloblastic leukemia. A common trait of HC, RS cells and B lymphocytes was the presence of surface receptors. A more precise classification system for the diagnosis of RS and RS cell leukemia is suggested along with the use of the term "hairy cell" to replace the misleading term "leukemic reticuloendotheliosis."

1626 PROLACTIN-SECRETING ADENOMAS: A LIGHT AND ELECTRON MICROSCOPICAL STUDY. (Eng.) Robert, F. (Notre-Dame Hosp., 1560 Sherbrooke St. East, Montreal, Quebec, Canada H2L 4K8); Hardy, J. *Arch. Pathol.* 99(12):625-633; 1975.

The light microscopic and ultrastructural findings in 25 prolactin-secreting pituitary adenomas removed by transsphenoidal surgery were analyzed and compared with previously published cases. Under the light microscope, these adenomas could easily be confused with chromophobe adenomas. However, in eight cases a small number of cells contained erythrosinophilic granules of the type found in prolactin cells. Electron microscopic study of the tumor cells showed scarce secretory granules frequently undergoing exocytosis, a prominent rough endoplasmic reticulum, and a large Golgi area containing immature granules. Cytoplasmic bundles and aggregates of filaments, as well as myelin figures and glycogen within the mitochondria were occasionally found. A comparison of these cells with previous reports of prolactin cells during lactation, in pituitary autografts, and under *in vitro* stimulation indicates that the tumor cells are actively secreting.

1627 MULTIPLE HORMONE PRODUCING ISLET CELL CARCINOMAS OF THE PANCREAS: A MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATION. (Eng.) Hammar, S. (Univ. Utah Coll. Med, Salt Lake City); Sale, G. *Hum. Pathol.* 6(3):349-362; 1975.

Biochemical, light, and electron microscopic studies of two multiple hormone producing metastatic islet cell carcinomas of the pancreas are presented. Both tumors initially produced symtoms referable to a single hormone and over a period of years produced two other endocrine active polypeptides. One case was a 62-yr-old white male with apparent Zollinger-

:llison syndrome. Biochemical analysis of the tumor howed 380 pg/g tumor tissue of glucagon, 196 mμg/g nsulin, and 811 ng/g gastrin. The other case was a 5-yr-old white female. Biochemical analysis of umor metastases in the liver showed 890 pg/g tumor issue of ACTH, 252 mμg/g insulin, and 1102 pg/g lucagon. The tumor in the first case had been stud-ed electron microscopically six years previously but emonstrated no significant ultrastructural changes ince then. This was in spite of clinical and bio-hemical evidence that the tumor had "progressed" o the formation of two new polypeptide hormones. he tumor in the second case contained secretory ranules of markedly varying size and density, sug-esting a formation of packaged precursor molecules f possibly all three hormones. It is proposed that hese tumors may arise from primitive neurectodermal ells, the substances produced depending upon the unctioning genes.

628 MIXED ENDOCRINE PANCREATIC TUMORS PRODUCING SEVERAL PEPTIDE HORMONES. (Eng.) Larsson, . I. (Dep. Histol., Univ. Lund, Sweden); Grimelius, .; Hakanson, R.; Rehfeld, J. F.; Stadil, F.; Holst, .; Angervall, L.; Sundler, F. *Am. J. Pathol.* 79(2): 71-284; 1975.

he different cell types in endocrine pancreatic tu-ors were demonstrated by immunohistochemistry and ight microscopy. Twenty-four islet cell tumors were emoved at surgery and subjected to indirect immuno-luorescence to demonstrate insulin, gastrin, glucagon, r ACTH. Antisera against these hormones were diluted nd incubated with tumor sections for 30 min. Con-rols included antisera inactivated by the correspond-ng hormone. For light microscopy, sections were tained with argyrophil, argentaffin, or aldehyde uchsin stains. Of the 24 tumors, six contained no ecognizable endocrine cell type, and 11 contained nly one. In these 11, clinical features were re-ated to hypersecretion of the hormone in question. n the remaining seven cases there were at least two ypes of peptide producing cells; there was no evi-ence of this clinically. Two contained insulin-nd glucagon-producing cells; two contained cells roducing gastrin, insulin, and glucagon; and one had ells producing gastrin and glucagon. One tumor had astrin-producing cells and cells which, under micro-pectrofluorometry, showed readings of serotonin. he remaining tumor had gastrin-producing cells and cell type unreactive to antisera that stained trongly by the Hellerstrom-Hellman argyrophil stain-ng technique. It is concluded that light microscop-c staining methods are unsatisfactory for classify-ng islet cell tumors, and that multihormonal tumors re more common than has been thought. Patients with ypersecretion of one pancreatic hormone should be xamined for hypersection of other peptide hormones.

629 HEPATITIS ASSOCIATED ANTIGEN, CIRRHOSIS AND PRIMARY CARCINOMA OF THE LIVER IN THE AMBIA. (Eng.) Ree, G. H. (Hosp. for Tropical Dis-ases, London, England). *Trans. R. Soc. Trop. Med. yg.* 69(2):263-265; 1975.

proportion of patients with liver disease who were admitted to a Gambian hospital in an 18-mo period were tested by gel diffusion for α_1-fetoprotein and by radioimmunoassay for hepatitis-associated antigen (HAA). Cirrhosis was diagnosed in 65 patients (38 men and 27 women) during the period of the investiga-tion and liver cancer in 63 (48 men and 15 women). Among the patients with hepatic carcinoma, 12 men and 2 women suffered from an associated cirrhosis. None of the 31 cirrhosis patients investigated was posi-tive for α_1-fetoprotein; 14 (45%) were positive for HAA. Of the 34 cancer patients tested, 18 (53%) were positive for HAA and 29 (85.3%) were positive for α_1-fetoprotein. Among the patients positive for α_1-fetoprotein, 16 were also positive for HAA. The results of this investigation lend further support to the etiologic role of hepatitis in primary liver can-cer.

1630 MORPHOLOGY OF LIVER DAMAGE AMONG POLYVINYL CHLORIDE PRODUCTION WORKERS. A REPORT ON 51 CASES. (Eng.) Gedigk, P. (Inst. Pathol., Univ. Bonn, West Germany); Müller, R.; Bechtelsheimer, H. *Ann. N.Y. Acad. Sci.* 246:279-285; 1975.

Fifty-one patients exposed to vinyl chloride for long periods of time, and examined at different intervals after their last exposure were studied. The gener-ally discrete and unspecific histological alterations of the liver were classified into five groups: (1) degenerative changes of the liver parenchyma, includ-ing single cell necrosis and fatty change, (2) adapt-ive responses in hepatocytes, (3) fibrosis, either of the portal tracts or intralobular perisinusoidal "net-like" fibrosis, (4) hyperplastic and proliferative hepatocytes, and (5) activated and proliferating si-nusoidal cells. In two cases of liver sarcoma, the demarcation of the proliferative activity of the si-nusoidal cells in the nontumorous part indicated that dilatation of the sinusoids may preceed the develop-ment of tumors; a transitional zone appeared to exist, showing hepatocytes with a marked hyperplasia and polyploidy, surrounded by active and proliferating sinusoidal cells. The tumors showed a marked varia-tion in morphological pattern, including anaplastic areas and cavernous structures. The degenerative areas were sharply delimited from the undamaged pa-renchyma. The unusual occurrence of activation and proliferation of the sinusoidal cells as well as of the hepatocytes may be characteristic of vinyl chlo-ride damage. Stronger degenerative changes resulted from longer exposure to vinyl chloride; their gradual disappearance suggests dose dependency and reversi-bility. Time of exposure also parallels the colla-genization of sinusoidal walls, septal fibrosis, and proliferation and hyperchromasia of sinusoidal cell nuclei; it was most frequent after ten yr of expo-sure, and was irreversible. Morphologically, arsenic damage and vinyl chloride injury bear a resemblance, and might be considered identical.

1631 CLINICAL MANIFESTATIONS AND COURSE OF VINYL CHLORIDE DISEASE. (Eng.) Veltman, G. (Inst. Exp. Hematol. Blood Transfus., Univ. Bonn, West Germany); Lange, C.-E.; Juhe, S.; Stein, G.; Bachner, U. *Ann. N.Y. Acad. Sci.* 246:6-17; 1975.

The test results of 70 employees of a polyvinyl

chloride (PVC) plant, plus information on further clinical changes after termination of vinyl chloride exposure, are presented. The patients had been primarily involved with cleaning autoclaves for an average of 7.7 yr. The clinical, scintigraphic, and radiological findings most frequently noted included thrombocytopenia (81%), increased BSP retention (67.2%), splenomegaly (57.4%), and reticulocytosis (41%). Skin changes initially consisted of clubbing-like swellings and shortenings of the terminal digital phalanges, followed by scleroderma-like skin changes, primarily above the metacarpophalangeal joints. Histological observations of test excisions taken from the hands of 36 patients showed distinct hyperorthokeratosis, a thinning of the epidermis, and rarefaction fissuration, and fragmentation of the elastic fibers; these were present in all clinically changed skin, and in 20% of the cases of unchanged skin. Changes in the bones included the characteristic band-like acroosteolysis in the bases of the processus unguiculares, small fragments of bones or sequestra in the absorbing ligaments, and subsequent advanced osteolysis in the proximal direction along the shaft. However, x-rays revealed improvement or complete restitution in some patients. Evident vascular changes revealed by arteriography ranged from slight narrowing of the vascular lumina in the digital arteries to demonstrable retardation of the filling process, strong stenosis, and subtotal occlusions. Improved platelet count was evident in only 20% after cessation of exposure, whereas bone and skin changes restitution tendencies were proved more frequent. The occurrence of thrombocytopenia was revealed in 81% of all patients; values ranged from 17,000-143,000/μl, whereas the lower norm is 150,000/μl. In view of the normal findings concerning bone marrow and the determination of platelet functions, thrombocytopenia probably results in increased platelet destruction in sites in addition to the spleen.

1632 RETICULOSARCOMATOSIS ORIGINATING FROM SKIN -- A CLINICOPATHOLOGICAL STUDY.

(Eng.) Okayasu, I. (Tokyo Med. Dent. Univ., Japan); Okayasu, N.; Mori, W.; Miyazaki, K.; Matsubara, S. *Acta Pathol. Jpn.* 25(2):201-212; 1975.

A correlation was found between the clinical findings and the pathomorphological changes of the cutaneous lesions of a case of reticulosarcomatosis cutis in a 65-yr-old man. There were mutliple tumors composed of reticulum cells located only in the skin, particularly around the large joints throughout the entire course of illness (three years and five months). Regression by irradiation, recurrence, or new formation of tumors were repeatedly seen. Systemic tumors of the skin and various visceral organs, including lymph nodes occurred shortly before death. From histological and electron microscopic examinations of biopsy and autopsy materials, it was ascertained that the tumor cells had become smaller in size. There were more pyknotic nuclei, and more scanty cytoplasm showing some pleomorphism. Small lymphocytes, seen in the tumor tissue, showed a gradual decrease in number throughout the clinical course. The changes in the specimens taken throughout the course of the disease may show some evidence of the change of the host's reaction against the tumor caused by tumor invasion into the blood vessels.

1633 WHY ARE OVARIAN TERATOMAS BENIGN WHILST TERATOMAS OF THE TESTIS ARE MALIGNANT?

(Eng.) Riley, P. A. (Univ. Coll. Hosp. Medical Sch., London WC1E 6FF, England); Sutton, P. M. *Lancet* (7921):1360-1362; 1975.

A mechanism is suggested to account for the fact that most ovarian teratomas are benign (dermoid cysts) while testicular teratomas are almost always malignant. The difference in the behavior of the two types of teratomas is explicable in terms of a genetic deletion affecting the growth-controlling locus in the gametocyte progenitor cells (germ cells). A recessive mutation affecting the spermatogonia would become homozygous at the first meiotic division, giving rise to a malignant tumor of secondary spermatocytes capable of generating many diverse tissue products. By contrast, a single recessive mutation affecting the control locus in primary oocytes may result, on completion of the first meiotic division, in a heterozygous condition; the phenotypic expression of this condition would be an abnormal proliferation of the cells without a total breakdown of tissue homeostatis--that is, a benign tumor. By differentiating and activating different growth-controlling loci, the secondary oocytes forming the benign ovarian teratoma avert the risk of malignant transformation.

1634 PRESACRAL TERATOMA IN THE ADULT. (Eng.)

Head, H. D. (Walter Reed Army Med. Cent., Wash., D.C.); Gerstein, J. D.; Muir, R. W. *Am. Surg.* 41(4):240-248; 1975.

Seventy-one documented cases of presacral teratomas in adults are analyzed, and two additional case studies are presented. A 70-yr-old Negro man presented with constipation and slight bulging of the posterior rectal wall. Rectal examination revealed a large, nontender bulging mass. Upon excision, the 10 x 7 x 5 cm mass was determined to be multiloculated and nonmalignant, with numerous optic structures. It was composed of stratified squamous and ciliated pseudostratified epithelium and it was diagnosed as benign cystic teratoma. The second case involved a 19-yr-old Caucasian female, initially presenting with intermittant sharp, stabbing pain in the lower right quadrant; a 5 x 5 cm nontender, nonpulsatile smooth fluctuant mass in the presacral area was subsequently excised. The mass was composed of white, keratinaceous liquid material containing hair; microscopic examination revealed numerous cystic spaces separated by a fibrous and smooth muscle stroma containing sweat and sebaceous glands, peripheral nerves and hair follicles, and stratified squamous and transitional epithelium. Again, the diagnosis was benign cystic teratoma. The study of 71 cases revealed that 63% of the patients were under 40 yr, 75% were females, and that 35% had a history of a tumor mass or cyst in infancy. The patients had presented most frequently (21 of 60 cases) with a mass located at the base of the spine or protruding in the

area of the buttock; draining fistulas and gastrointestinal symptoms occurred in four cases. Roentgenographic studies revealed calcification or bone formation in 11 of 39 cases, and sacrococcygeal anomalies occurred in four of these patients. Barium enema, myelography, and ultrasonography may all be useful diagnostic procedures. Eleven percent of the presacral tumors were malignant, and resulted in death. Recurrences after total surgical excision of the teratoma is rare.

1635 HYPERPARATHYROIDISM AND CARCINOID TUMOR. (Eng.) Samaan, N. A. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.); Hickey, R. C.; Bedner, T. D.; Ibanez, M. L. *Ann. Intern. Med.* 82(2):205-207; 1975.

Case histories of three patients with hyperparathyroidism and carcinoid tumor of the gut were reviewed to determine possible association between the two conditions. All patients showed a high level of immunoreactive calcitonin in peripheral circulation with peaks ranging between 1.5 ng/ml and 2.4 ng/ml during calcium infusion, but with no differential increase in neck venous catheterization specimens. There was no evidence of medullary carcinoma of the thyroid upon surgery or post-mortem examination. Following surgical removal of the parathyroid adenoma in two of the patients, hypercalcemia disappeared, indicating that the calcitonin level was unrelated to the hypercalcemia. Hyperparathyroidism appeared likely therefore, to have been primary in these patients. The association with carcinoma may represent another form of multiple endocrine tumor formation due to dysplasia of the neural ectoderm. Investigation of hyperparathyroidism in patients with carcinoid tumor is indicated.

1636 BREAST CANCER, PROSTAGLANDINS, AND BONE METASTASES. (Eng.) Bennett, A. (King's Coll. Hosp. Med. Sch., London, England); McDonald, A. M.; Simpson, J. S.; Stamford, I. F. *Lancet* 1 (7918):1218-1220; 1975.

1637 MUCUS-PRODUCING CARCINOMA OF THE BREAST -- REPORT OF 6 CASES. (Jpn.) Narai, S. (Univ. Niigata Sch. Med., Niigata, Japan); Soga, J.; Sano, M.; Karaki, Y. *Gan No Rinsho* 21(11): 929-934; 1975.

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1639 BREAST CANCER IN BROTHERS: CASE REPORTS AND A REVIEW OF 30 CASES OF MALE BREAST CANCER. (Eng.) Marger, D. (112 Bethpolamy Court, Dayton, Ohio 45415); Urdaneta, N.; Fischer, J. J. *Cancer* 36(2):458-461; 1975.

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1645 PARTIALLY RESECTED AND IRRADIATED CEREBELLAR ASTROCYTOMA OF CHILDHOOD: MALIGNANT EVOLUTION AFTER 28 YEARS. (Eng.) Budka, H. (Neurol. Inst. Univ. Vienna, Austria). *Acta Neurochir. (Wien)* 32(1/2):139-146; 1975.

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1662 UNUSUAL ULTRASTRUCTURAL FEATURES OF A LEIOMYOSARCOMA OF THE LUNG. (Eng.) Pritchett, P. S. (Med. Coll. Virginia, Richmond); Fu, Y.-s.; Kay, S. *Am. J. Clin. Pathol.* 63(6):901-908; 1975.

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22901); Rosenthal, J. D.; Shaw, A. *Am. J. Dis. Child.* 129(6):742-743; 1975.

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1678 A CASE OF ACUTE LYMPHOBLASTIC LEUKEMIA AND THOROTRAST-ACCUMULATION. (Eng.) Jobets, M. A. (Ludwig-Boltzmann-Inst. für Leukämieforschung und Hämatologie, Hanusch-Krankenhaus, Wien, Austria); Nowotny, H.; Ruzicka, F.; Hanak, H.; Stacher, A. *Blut* 31(1):5-10; 1975.

1679 CYTOCHEMICAL STUDY OF ACUTE PROMYELOCYTIC LEUKAEMIA. (Eng.) Liso, V. (Univ. Bari Med. Sch., Italy); Troccoli, G.; Grande, M. *Blut* 30(4):261-268; 1975.

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1681 TRANSLOCATION OF THE PHILADELPHIA CHROMOSOME ONTO THE 17 SHORT ARM IN CHRONIC MYELOID LEUKEMIA: A SECOND EXAMPLE [letter to editor]. (Eng.) Engel, E. (Vanderbilt Univ. Sch. Medicine, Nashville, Tenn.); McGee, B. J.; Flexner, J. M.; Krantz, S. B. *N. Engl. J. Med.* 293(13): 666-667; 1975.

1682 MINUTE CHROMATIN BODIES IN A MURINE *IN VITRO* CELL LINE. (Eng.) de Salum, S. B. (Instituto de Investigaciones Hematologicas, Academia Nacional de Medicina, J. A. Pacheco de Melo 3081, Buenos Aires, Argentina); Larripa, I. *J. Natl. Cancer Inst.* 55(3):717-720; 1975.

1683 PRIMARY FIBROSARCOMA OF THE LIVER: CASE REPORT AND REVIEW OF THE LITERATURE: (Eng.) Alrenga, D. P. (Cook County Hosp., 1825 W. Harrison St., Chicago, Ill. 60612). *Cancer* 36(2): 446-449; 1975.

1684 VARIATION OF SPREAD OF OVARIAN MALIGNANCY ACCORDING TO SITE OF ORIGIN. (Eng.) Meleka, F. (Methodist Hosp., 506 Sixth St., Brooklyn, N.Y. 11215); Rafla, S. *Gynecol. Oncol.* 3(2): 108-113; 1975.

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1688 TESTICULAR TUMOURS IN BROTHERS-IN-LAW [letter to editor]. (Eng.) MacLaren, R. G. C. (Ontario Cancer Foundation, Hamilton Clinic, Hamilton, Ont., Canada). *Can. Med. Assoc. Med. J.* 113(6):498; 1975.

1689 SEMINOMA IN A RENAL TRANSPLANT RECIPIENT. (Eng.) Nellans, R. E. (Dep. Surg. (Urol.), Univ. California, Irvine); Ravera, J. *J. Urol.* 113(6):871-873; 1975.

1690 MALIGNANT TERATOMA IN THE ADULT THYROID GLAND. (Eng.) O'Higgins, N. (Hammersmith Hosp., London, England); Taylor, S. *Br. J. Clin. Pract.* 29(9):237-238; 1975.

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1692 AN UNUSUAL LEIOMYOSARCOMA OF THE UTERUS CONTAINING OSTEOCLAST-LIKE GIANT CELLS. (Eng.) Darby, A. J. (Middlesex Hosp. Medical Sch., Ridinghouse St., London, WIN 8AA, England); Papadaki, L.; Beilby*, J. O. W. *Cancer* 36(2):495-504; 1975.

1693 SQUAMOUS NEOPLASIA OF VAGINA RELATED TO DES SYNDROME. (Eng.) Fetherston, W. C. (St. Mary's Hosp., Milwaukee, Wis.). *Am. J. Obstet. Gynecol.* 122(2):176-181; 1975.

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1695 URETERAL CARCINOMA *IN SITU*. (Eng.) Linker, D. G. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.); Whitmore, W. F. *J. Urol.* 113(6):777-780; 1975.

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1697 BILATERAL WILMS' TUMOUR: AGE AT DIAGNOSIS, ASSOCIATED CONGENITAL ANOMALIES, AND POSSIBLE PATTERN OF INHERITANCE. (Eng.) Bond, J. V. (Inst. Child Health, London WC1N 1EH, England). *Lancet* 2(7933):482-484; 1975.

1698 INCREASED TUMOR METASTASIS AFTER *IN VITRO* ALTERATION OF THE CELL SURFACE. (Eng.) Parks, R. C. (American Medical Center Denver, Spivak, Colo. 80214). *J. Natl. Cancer Inst.* 54(6): 1473-1474; 1975.

1699 MORPHOLOGIC AND MICROSPECTROPHOTOMETRIC STUDIES ON SPONTANEOUS MELANOMAS IN *XIPHOPHORUS HELLERI*. (Eng.) Ishikawa, T. (Cancer Inst., 1-37-1 Kamiikebukuro, Toshima-ku, Tokyo 170, Japan); Sakakibara, K.; Kurumado, K.; Shimada, H.; Yamaguchi, K. *J. Natl. Cancer Inst.* 54(6):1373-1378; 1975.

See also:

* (Rev): 1215, 1216, 1217, 1218, 1223, 1230, 1235, 1243, 1244, 1245, 1246, 1247, 1248, 1249, 1255
* (Chem): 1276, 1283, 1292, 1302, 1303, 1315, 1317, 1322, 1324
* (Phys): 1365, 1370, 1372
* (Immun): 1485, 1487, 1544, 1553
* (Epid-Biom): 1704, 1714, 1720

1700 EPIDEMIOLOGIC CHARACTERISTICS OF PATIENTS WITH PROSTATIC NEOPLASMS. *(Eng.)* Armenian, H. K. (Johns Hopkins Univ., Sch. Hygiene and Public Health, 615 N. Wolfe St., Baltimore, Md. 21205); Lilienfeld, A. M.; Diamond, E. L.; Bross, I. D. J. *Am. J. Epidemiol.* 102(1):47-54; 1975.

The epidemiology of prostatic neoplasms was studied in 128 patients with benign prostatic hyperplasia (BPH) and in 290 prostate cancer patients; 256 and 290 age-matched controls for the BPH patients and the cancer patients, respectively, were observed. The group was selected from interviews and questionnaires administered to 95% of all patients admitted to Roswell Park Memorial Institute between 1957 and 1965. Percentage distributions of cases and controls were calculated for characteristics of interest. The prostate cancer patients had a larger proportion of people with longer marriages and their wives had a larger mean number of pregnancies compared to the control and BPH groups. Thirty-five BPH cases who developed prostate cancer were matched with BPH cases who did not; a larger percentage of the patients developing prostate cancer had offspring (91.4% compared to 77.1%). Married patients with children had a 7.0% risk for prostate cancer; this was 2.69 times the risk of those without children (2.6%). Positive findings were limited to the prostate cancer group. Differences in fertility between prostate cancer patients and their controls may reflect constitutional hormonal differences predisposing them to higher fertility and prostate cancer.

701 EVALUATION OF MORTALITY DATA FOR CERVICAL CANCER WITH SPECIAL REFERENCE TO MASS SCREENING PROGRAMS, DENMARK, 1961-1971. (Eng.) rünfeld, K. (Danish Inst. Clin. Epidemiol., Copenagen); Horwitz, O.; Lysgaard-Hansen, B. *Am. J. pidemiol.* 101(4):265-275; 1975.

n epidemiological study of cervical cancer was underken to assess the effect of a mass screening proram in operation since 1968 on women aged 40-49 yr d living in Denmark's capital. Between 1961-1971, 96 women over 25 died from cervical cancer. The nual mortality per 1000 women was 0.23, which reined constant during the period. The mortality in e Capital was 43% higher than in the Provinces. en it was taken into account that there are more d women and unmarried women in the Capital, a true cess of 20% remained. Mortality was lowest among ung women, below 0.02 per 1000, with an increase rate up to age 45. Single women had the lowest rtality; among married women it was doubled, among dows it was 2-3 times higher, and among divorcees was increased four-fold. The mortality rate avaged 31% higher for single women in the Capital an in the Provinces. Married women had 35% higher rtality in the Capital, and widows had 25% higher. vorcees averaged 20% lower in the Capital. The vest socioeconomic groups had the highest mortal- at young ages. The effects of a "mass" (total oulation) versus a "selective" (age group 40-59 yr) eening program were estimated by calculating the iber of cervical cancer deaths which would be preted annually if a screening program could reduce the mortality by 10%, 50%, or 100%. If "selective" screening were 100% effective, the number of deaths prevented would be half of that obtained in a "mass" program. By relating the hypothetical prevented deaths to all cancer deaths and to deaths from all causes, the impact on public health was evaluated. If cervical cancer were completely eradicated, the total cancer mortality would drop by 5.9%. The female population was subdivided by age, marital status and residence and ranked according to cervical cancer mortality. These data were used to design hypothetical programs which would minimize the number of examinees and maximize the number of prevented deaths, thereby improving on the present program.

1702 EPIDEMIOLOGY OF ADULT LEUKAEMIAS IN PUNJAB DURING PAST SIX YEARS (1967-1972). *(Eng.)* Singh, P. (Victoria Jubilee Hosp., Amritsar, Punjab, India); Singh, H. *J. Assoc. Physicians India* 23(3):193-200; 1975.

An epidemiological study was aimed at detecting associations of cases of leukemia in time and space in 603 during 1967-1972 in the adult population of Punjab State and Chandigarh in India. All types of leukemia cases were included that sought admission to five hospitals after the 13th birthday. Date of onset, address at time of onset and reports of peripheral blood counts, blood films, bone marrow examination and clinical findings were analyzed for clustering in time and space. Cases were plotted on district maps, measuring the distance between two similar cases in the same year. No tendency for high or low incidence areas to lie close to one another was observed. There were no pairs found of the same type leukemia 60-90 days apart. From 1967-71, there was neither increased frequency in any year, nor any peak concentration of cases monthly or seasonally. Results of this study do not support the hypothesis that childhood leukemias occur in clusters together in space or time.

1703 EPIDEMIOLOGICAL STUDIES ON FELINE LEUKAEMIA VIRUS INFECTION. I. A SEROLOGICAL SURVEY IN URBAN CATS. (Eng.) Rogerson, P. (Univ. Glasgow Vet. Sch., Scotland); Jarrett, W.; Mackey, L. *Int. J. Cancer* 15(5):781-785; 1975.

A survey of the incidence of feline leukemia virus (FeLV) infection in cats in a large urban area was made by studying the prevalence of antibodies to FeLV-associated cell membrane antigens. Of 200 cats studied, 150 were pets and 50 were strays. Immunofluorescence and a mixed immunoglobulin rosette technique were used. The overall incidence of cats with antibodies was 40%, contrasting with 6% in the surrounding rural area. Only 6% of urban kittens were positive, compared to 50% of the adults. The incidence in adults rose from 29% at 5-6 mo to 74% in cats over three yr. Stray cats had an incidence twice that of domestic pets. These results support and extend earlier findings that FeLV infection is common and is horizontally transmitted.

1704 COLORECTAL CANCER IN CHILDREN: EPIDEMIOLOGIC ASPECTS. (Eng.) Chabalko, J. J. (Natl. Cancer Inst., Bethesda, Md.): Fraumeni, J. F., Jr. *Dis. Colon Rectum* 18(1):1-3; 1975.

An epidemiologic survey was made from nationwide mortality data and a multihospital case review to characterize colorectal cancer in children. The mortality survey included 50 children less than 15 yr old who died of colorectal cancer during 1960-68 and 76 children from 15-19 yr old who died during 1965-68. Information from the hospital survey was obtained from 76 patients with colorectal cancer, 34 of which were less than 20 yr old. The rare childhood form of the cancer differed in a few respects from the adult disease. Childhood mortality (from birth to 19 yr) was greater among Negroes (boys--5.02/10^6/yr and girls--1.32/10^6/yr) than Caucasians (boys--1.24/10^6/yr and girls--0.70/10^6/yr), particularly in boys. This reflects the rising incidence of this tumor in the young Negro population and suggests an environmental influence. The percentage (10.5%) of childhood cases with precancerous diseases (polyposis, colitis) was higher than in adults. A high percentage (24/76) of mucin-producing tumors in the young with colorectal cancer was found. The mucoid tumors usually occurred after the age of ten yr. Younger children were more likely (9/13 children) to develop nonmucoid carcinoma in an adenomatous polyp. Thus, mucin-producing adenocarcinoma and adenocarcinoma developing in a polyp have different age patterns in childhood and possible different etiologic mechanisms.

1705 SOCIAL CLASS DIFFERENCES AMONG PATIENTS WITH LARGE-BOWEL CANCER IN CALI, COLOMBIA. (Eng.) Haenszel, W. (Natl. Cancer Inst., Bethesda, Md.); Correa, P.; Cuello, C. *J. Natl. Cancer Inst.* 54(5):1031-1035; 1975.

The occurrence of large-bowel cancer in Cali, Colombia, as it relates to social differences, was studied. Cali natives diagnosed as having adenocarcinoma of the large bowel were divided into four socioeconomic classes (I, upper; II, middle; III, low; and IV, very low). The results are presented as standardized incidence ratios obtained by application of the age- and sex-specific incidence rates for this area to corresponding populations for each group of census tracts to compute expected number of cases. Incidence data were supplemented with findings on social class differences in prevalence of intestinal polyps found in Cali residents. Measurements made on fresh colon specimens taken from individuals who died from accidental causes were used to examine the relationship between tissue at risk and cancer by segment. After reference points were marked, the colon was removed and placed in water at atmospheric pressure followed by the removal of fat from the serosa. There was a significant increase in the number of large-bowel cancers in Classes I and II (in both men and women) over that of Classes III and IV. An excess risk for Classes I and II was found to be confined to segments between the ascending and rectosigmoid colon, with the social class gradient for sigmoid and immediately adjacent segments more strongly expressed in males. Cancer of the cecum and rectum presented no statistically significant social class gradient in risk. It is suggested that dietary intake and standards for living has a great influence on large-bowel cancers occurring in a single community. Further investigations involving high-risk populations are indicated.

1706 CARCINOMA OF THE LARGE BOWEL IN THE SUDAN. (Eng.) Elmasri, S. H. (Dep. Surg., Univ. Khartoum, Sudan); Boulos, P. B. *Br. J. Surg.* 62(4): 284-286; 1975.

Histopathologic reports of 272 specimens of carcinoma of the large bowel received in the Central Research Laboratory, Khartoum from 1963-1972 were reviewed and analyzed. Squamous cell carcinoma of the anal canal was included in the analysis, and was found to be of a higher incidence than is reported for Western countries. Of 6,725 cancers reported in the Sudan during this period, carcinoma of the large bowel accounted for 4.04%; this represents an increase over previous reports. No example of polyposis, ulcerative colitis, or coexisting benign polyps was found. One specimen of bilharzial granuloma with ova surrounded by squamous cell carcinoma was reported. Of the carcinomas of the large bowel, 203 specimens were located in the anorectum and 69 specimens were found in the colon. Despite the site of the lesion, the ratio of males to females remained constant at 5:3 (167:105). An average age of 44.5 yr was reported for cancer of the colon; 85% of the persons were below the age of sixty. For anorectal cancers the average age was 52.3 yr with 78% under sixty. Furthermore, 16.5% of the anorectal cancer patients were below the age of 30 while 14% of the patients with colonic lesions were under this age. The authors suggest that this early incidence may be associated with the high proportion (29%) of undifferentiated and mucoid types of carcinoma. Only two of the cancers studied were from the Southern Sudan, where the transit time in the large bowel has been shown to be shorter than for the Northern Sudan. The authors conclude that diet alone cannot account for this geographic distribution. Other factors relating to socioeconomic development must be examined to explain the increasing incidence of cancer of the large bowel in the Sudan.

1707 ALIMENTARY TRACT CARCINOMAS IN NIGERIAN IGBOS. (Eng.) Onuigbo, W. I. B. (Gen. Hosp., Enugu, Nigeria). *Arch. Surg.* 110(3):349; 1975

Twenty cases of alimentary tract carcinoma were found in about 5,000 surgical specimens obtained from Nigerian Igbo tribesmen between 1970-1974. Average age was 47.4 yr and men outnumbered women 16 to 4. Esophageal carcinoma was not seen and polyps and diverticula were absent in the nonmalignant parts of the specimens. Alimentary tract, and especially esophageal cancers, are apparently rare among members of this tribe. Study of the Igbo's bulky carbohydrate diet as a possible explanation is suggested.

708 THE CHANGING PATTERN OF CANCER MORTALITY IN SOUTH AFRICA, 1949-1969. *(Eng.)* Bradshaw, E. (Cancer Res. Unit Natl. Cancer Assoc. South Africa, South African Inst. Medical Res., Johannesburg, South Africa); Harington, J. S. *S. Afr. Med. J.* 49(23):919-925; 1975.

Cancer mortality rates for South African Caucasians, Negroes and Asians were determined from death certificates from 1948-1969. The annual population-at-risk was calculated from censuses and the number of deaths per 100,000 population in each race-sex-age group was calculated. Mortality rates were plotted over time for the three groups studied. Regression lines were calculated for each curve and only a change in rate of 95% confidence limit is referred to as significant. In males, cancers of the stomach, lung and prostate caused half the cancer deaths in all three races. In females, cancers of the stomach, breast, cervix and uterus were most common. Lung cancer rates rose in all three male groups. Cancer of the esophagus is the most common site of cancer in Negro males. White women had the highest rate of breast cancer and black women had the highest rate of cervical cancer. Cancer of the prostate was higher in Caucasian males than Negroes. South African males have the fourth highest rate of stomach cancer in the world. Overall cancer risk varies among race groups, being highest for Negro males and lowest for Asian males. Most common cancers are not the same in each group, nor do they remain the same over a long period.

709 CANCER INCIDENCE IN THE MEXICAN-AMERICAN. (Eng.) Menck, H. R. (Univ. Southern California Sch. Medicine, 2025 Zonal Ave., Los Angeles, Calif. 90033); Henderson, B. E.; Pike, M. C.; Mack, .; Martin, S. P.; SooHoo, J. *J. Natl. Cancer Inst.* 5(3):531-536; 1975.

verage annual age-specific incidence rates for all ancer sites and selected sites were calculated for ale and female Spanish-surnamed residents of Los ngeles County and for other whites. For all sites ombined, male adult immigrants and middle-aged inigenous Mexican-Americans experienced lower rates han other whites. For females, the differences ppeared only at older ages. The Mexican-Americans ere at lower risk for cancer of the buccal cavity, olon, rectum, larynx, lung, breast, bladder, prosate, and testis. However, they were at higher risk or cancer of the stomach, gallbladder, liver and ervix. Immigrant Mexican-Americans had incidence ates most divergent from other whites, whereas ingenous Mexican-Americans had rates between the her two groups. The cancer pattern in Mexicanericans was generally similar to that in American dians. Although genetic factors may be partially sponsible for some of the differences between Mexan-Americans and other whites, the data are most nsistent with environmental causal variations.

10 AN EXPERIENCE OF CANCER: A TEN-YEAR SURVEY OF MALIGNANT DISEASE ARISING IN SCOTTISH ISLAND. *(Eng.)* Crosfill, M. L. (Lewis sp., Stornoway, Scotland). *Br. J. Clin. Pract.* (6):137-142; 1975.

A study of the incidence of malignant disease arising in the single island of Lewis and Harris, located off the coast of Scotland, was made during 1960-69. Genetic isolation of the population (24,000) was assumed. Cancer indicence was obtained from the registers of local hospitals. The final total of cancer patients was 863. The incidence of lip carcinoma in this population is seven times higher than that in Scotland and 14 times that in Britain. The author suggests that too little attention has been paid to genetic factors involved in the genesis of epithelioma of the lip. A high incidence of carcinoma of the thyroid was also discovered between 1958 and 1972. The rates were 4.6 per 100,000 per yr in males and 7.0 in females on the island, as compared to 0.9 males, 2.4 females; and 0.7 males, 1.8 females in Scotland and England, respectively. A survival curve was developed on the basis of a five-yr survival rate in patients after "curative operations" were performed for carcinoma of the breast, prostate, colon and rectum. Survival rates ranged from 14-39% for patients with major cancers on Lewis and Harris. The author concludes that the results of treatment are comparable to results elsewhere.

1711 LUNG CANCER AND SMOKING IN DANISH WOMEN. *(Eng.)* Jensen, O. M. (The International Agency for Res. on Cancer, Unit of Epidemiology and Biostatistics, 150, cours Albert Thomas, 69008-Lyons, France). *Int. J. Cancer* 15(6):954-961; 1975.

Time trends in lung cancer in Denmark from 1931-1972, and the relationship between lung cancer mortality and cigarette smoking in Danish women were reviewed. Data was collected entirely from mortality registers. A multi-stage sampling technique was used against the adult Danish general population resulting in a 3% sample of the general population. The data reveals an increase in female rates as opposed to a 16-fold increase in male death rates due to lung cancer. Since 1960, however, the female mortality rate continued to rise, leading to a decline in the male-female ratio of lung cancer mortality. Recent female cohorts in the sample contained an increased proportion of smokers and exhibited an increased risk of dying from lung cancer when compared to older cohorts. The proportion of cigarette smokers among all smokers fell from 98% in the youngest to 33% in the oldest age group. Among women in Denmark, an increased risk of lung cancer has been paralleled by increased cigarette consumption. Although no causal relationship between cigarette smoking and lung cancer can be postulated on this evidence along, the present sex difference in lung cancer rates may be a dose-response effect.

1712 THE CURRENT MORTALITY RATES OF RADIOLOGISTS AND OTHER PHYSICIAN SPECIALISTS: DEATHS FROM ALL CAUSES AND FROM CANCER. (Eng.) Matanoski, G. M. (Johns Hopkins Univ. Sch. Hyg. Public Health, Baltimore, Md.); Seltser, R.; Sartwell, P. E.; Diamond, E. L.; Elliott, E. A. *Am. J. Epidemiol.* 101 (3):188-198; 1975.

Cohort mortality experience of male radiologists,

physicians, otolaryngologists and ophthamologists (members of the Radiological Society of North America (RSNA), the American College of Physicians and the American Academy of Opthalmology and Otolaryngology, resp.) was examined over a 50-yr period to compare the differences among these groups relative to a presumed decrease in radiation exposure. Cause of death was determined from death certificates (98.1% of the cases) and obituaries from the *Journal of American Medical Association* (1.4%). The remaining 0.5% were classified as all-cause mortality since the cause of death was unknown. All-cause mortality rates were highest in radiologists for all cohorts (members entering a society within the same ten-yr period) of the RSNA before 1940, even when cancer deaths were removed from the rates. This was consistent with the concept of accelerated aging due to radiation. Cancer mortality for radiologists was higher than other specialists through 1949. The 1950-1959 cohort had not sufficiently aged to demonstrate the expected cancer peak mortality in the 60-64 age group. It is suggested that mortality rates vary with radiation exposure levels. Several hypotheses for the differences in age-specific cancer mortality rates are presented.

1713 CLINICAL MANAGEMENT OF WORKERS EXPOSED TO VINYL CHLORIDE AND POLYVINYL CHLORIDE. (Eng.) Johnson, C. A. (Goodyear Tire and Rubber Co., Akron, Ohio 44316). *Ann. N.Y. Acad. Sci.* 246:313-319; 1975.

A medical surveillance program has been introduced in two polyvinyl chloride production plants in an effort to detect occupational health problems early enough to prevent serious illness. The medical surveillance program consists of the following: (a) a comprehensive medical history including family history, past and present medical history, and occupational history; (b) physical examination by a physician; (c) chest X-ray; (d) X-ray of both hands; (e) pulmonary function test; and (f) clinical laboratory procedures consisting of hemoglobin and hematocrit determinations, a WBC count and differential, a platelet count, a blood chemistry profile, urinalysis, and a urine cytology. This examination is conducted on an annual basis and was approved by both management and union officials. Medical surveillance alone cannot be effective in the prevention of occupational disease, and the cooperative efforts of the toxicologist, the process engineer, industrial hygienist, physician, and worker are also required. A discussion of various aspects of the polyvinyl chloride occupational health problem follows this report. The discussion includes the views of a representative of a chemical workers union, several U.S. government officials, and researchers in the field.

1714 PROPORTIONAL MORTALITY AMONG VINYL CHLORIDE WORKERS. (Eng.) Monson, R. R. (Harvard Sch. Public Health, Boston, Mass. 02115); Peters, J. M.; Johnson, M. N. *Environ. Health Perspect.* 11:75-77; 1975.

In a proportional mortality analysis of 161 deceased workers in two plants processing or producing vinyl chloride, a 50% excess in deaths due to cancer was observed. In addition to four previously reported fatal cases of angiosarcoma of the liver, a fifth case was identified. Specific cancer sites with the greatest excess included liver and biliary tract, lung, and brain. The number of observed deaths from cancer of these sites was 8, 13, and 5, respectively, as opposed to an expected number of 0.7, 7.9, and 1.2. The excess in fatal cancer was seen mainly in men who died before age 60. Also, the ratio of observed to expected deaths increased with time. Prior to 1975, no excess of deaths due to cancer was observed, while since 1970 over twice as many cancer deaths as expected have occurred.

1715 VINYL CHLORIDE EXPOSURE IN A CONTROLLED INDUSTRIAL ENVIRONMENT: A LONG-TERM MORTALITY EXPERIENCE IN 594 EMPLOYEES. *(Eng.)* Ott, M. G. (Dow Chemical Co., Corporate Medical Dept., 2030 Dow Center, Midland, Mich. 48640); Langner, R. R.; Holder, B. B. *Arch. Environ. Health* 30(7): 333-339; 1975.

The relation between tumor incidence and the highest levels of vinyl chloride exposure was examined. Measurements of the work environment began in 1950 by collecting breathing zone samples on silica gel and measuring chlorides by the Volhard method to calculate vinyl chloride. More recently, a combination of gas chromatography and mass spectrometry has been used to identify airborne material. The study population included production employees who worked between 1942 and 1960 in areas of potential vinyl chloride exposure. Each job was classified according to time-weighted average (TWA) concentration of vinyl chloride for an eight-hr day. The high level concentration was defined as over 200 ppm vinyl chloride and the low level was below 25 ppm. Observed deaths among vinyl chloride workers were 91% of the expected deaths based on the U.S. white male population. Distribution of malignant neoplasms suggests a possible dose-response relationship, since nine of 13 malignancies were observed in the high exposure group. The χ^2 comparison of the distribution of malignancy deaths between high and all other exposure groups was significant ($P < 0.01$). No adverse malignancy effects are demonstrated in the lower exposure categories.

1716 ENVIRONMENTAL ASPECTS OF FIBROUS GLASS PRODUCTION AND UTILIZATION. *(Eng.)* Dement, J. M. (Natl. Inst. for Occupational Safety and Health, Div. of Field Studies and Clinical Investigations, Cincinnati, Ohio 45202). *Environ. Res.* 9(3):295-312; 1975.

Investigations were made at four facilities manufacturing large diameter (fibers > 1 μm) insulation products and six facilities using small diameter (fibers < 1 μm) glass fibers. Fibrous glass exposure levels, especially respirable fibers of small diameter, were determined. Airborne fibrous glass samples were taken for evaluation of total airborne dust (mg/m^3) and fiber (fibers/ml) concentrations. Dust samples were collected on 37 mm polyvinyl chloride filters and fiber samples

ere collected on Millipore; fiber counts were ade using phase contrast microscopy. Airborne iber concentration in facilities involving large iameter glass fibers ranged from 0.000-0.83 fibers/ l; airborne dust concentrations ranged from 0.1- .5 mg/m^3. In operations producing or using small iameter glass fibers, airborne concentrations of ust were 0.1-2.0 mg/m^3 and fibers ranged from 0.1- .1 fibers/ml. Average airborne fiber diameter d length were also determined in operations in- lving small diameter glass fibers. A respirable iber was defined as less than 3.5 μm in diameter d less than 50 μm in length. The most respirable bers occur in operations manufacturing and using all diameter glass fibers.

17 ARSENICAL AIR POLLUTION AND LUNG CANCER. *(Eng.)* Blot, W. J. (Natl. Cancer Inst., 21 Landow Building, Bethesda, Md. 20014); Frau- ni, J. F., Jr. *Lancet* 2(7926):142-144; 1975.

ncer mortality-rates were calculated for the Cau- sian population in 71 primary smelting and re- ning (S.R.) counties in the United States. The R. counties were defined according to ore pro- ssed: (1) copper, lead, or zinc, which released organic arsenic during processing, and (2) alu- num or nonferrous metals, which contain small ounts of arsenic. Regression equations were used compute a standardized mortality ratio. Lung ncer mortality was significantly higher among les ($P < 0.001$) and females ($P < 0.05$) in the S.R. counties with copper, lead, and zinc in- stries, than in the other 2,985 counties of the ited States. Lung cancer mortality was not in- eased in the population from counties processing nferrous ores. The most likely reason for higher ng cancer mortality is air pollution from indus- ial sources of inorganic arsenic.

18 DESTRUCTION OF AFLATOXINS IN PEANUT PRO- TEIN ISOLATES BY SODIUM HYPOCHLORITE. ng.) Natarajan, K. R. (Food Protein Res. Dev. nt., Texas A&M Univ., College Station); Rhee, C.; Cater, C. M.; Mattil, K. F. *J. Am. Oil em. Soc.* 52(5):160-163; 1975.

dium hypochlorite was tested for destruction of xins in peanuts. Effects of sodium hypochlorite ncentration, reaction pH, temperature and time re studied. Split peanuts were blanched mechan- lly, and ground. The ground raw peanuts con- ined 725 ppb aflatoxin B_1 and 148 ppb aflatoxin . Peanut protein isolates were prepared from ground raw peanuts by an aqueous extraction ocess. In the case of raw peanuts, a calculated unt of 5% sodium hypochlorite solution was added the aqueous suspension at 60 C after adjusting pH 8, 9, or 10. In the case of peanut meal, raction was carried out at room temperature er the addition of the sodium hypochlorite. treatments were evaluated by determination of aflatoxins in the products by thin layer chro- ography. Treatment with 0.4% sodium hypochlorite pH 8 produced protein isolates with trace amounts aflatoxins B_1 and B_2 from ground raw peanuts, whereas untreated protein isolates contained 384 ppb aflatoxin B_1 and 76 ppb aflatoxin B_2. At pH 9, 0.3% sodium hypochlorite reduced the aflatoxin B_1 content in the protein isolates from 300 ppb to below detectable quantities and the aflatoxin B_2 content from 52 ppb to 2 ppb. Similar results were obtained at pH 10 for 0.3% sodium hypochlorite concentration. In the case of defatted peanut meal, which contained 136 ppb aflatoxin B_1 and 36 ppb aflatoxin B_2, 0.25% sodium hypochlorite concentra- tion at pH 8 (0.20% at pH 9; 0.15% at pH 10) reduced both the aflatoxin B_1 and B_2 contents to below de- tectable quantities in protein isolates as compared to aflatoxin levels of about 75 ppb B_1 and 17 ppb B_2 in the untreated protein isolates. Reaction temperature and time did not affect the destruction of aflatoxins significantly. Results showed that both the sodium hypochlorite concentration and pH are important factors in reducing the concentration of aflatoxins in the protein isolates to nondetect- able levels. The results suggest that it would be possible to destroy aflatoxin to nondetectable levels or to reduce the aflatoxin level in the protein isolates prepared from either raw peanut or defatted peanut meal to within acceptable levels.

1719 MOUSE MYELOMA: A MODEL FOR STUDIES OF CELL KINETICS. (Eng.) Bergsagel, D. E. (Ontario Cancer Inst., Toronto, Canada); Ogawa, M.; Librach S. L. *Arch. Intern. Med.* 135(1):109-113; 1975.

The growth characteristics of Adj PC-5 mouse myeloma cells are used in this model for the study of mechan- isms which determine the specificity of chemothera- peutic agents for tumor cells. The toxicities of alkylating agents for myeloma cells and for hemopoie- tic colony-forming cells are compared. Fourteen days after mice were injected i.v. with 10^7 tumor-infil- trated spleen cells, tritiated thymidine was injected. A high labeling index indicated the presence of a ma- jority of proliferating myeloma cells, but tumor growth was slow due to a large loss of cells into non- proliferative end-stage cells. An estimated 2.5%-5% of the myeloma cells were colony-forming stem cells and all of these were apparently in different stages of the cell cycle. Marked tumor specificity of sev- eral alkylating agents could not be explained by dif- ferences in proliferative states of myeloma cells and of normal marrow cells. Specificity is thus based on intrinsic differences between myeloma and normal cells. The differences in sensitivity of various mye- lomas to a single agent are probably related to struc- ture of the drug and to intrinsic properties of the cell, rather than to the agent's mechanism of action.

1720 KINETICS OF CELL PROLIFERATION IN BENIGN AND PREMALIGNANT TUMORS OF THE HUMAN EPIDERMIS. (Eng.) Heenen, M. (Sch. Med., Free Univ. Brussels, Belgium); Lambert, J. C.; Achten, G.; Galand, P. *J. Natl. Cancer Inst.* 54(4):825- 827; 1975.
The kinetics of cell proliferation in benign and premalignant tumors of human epidermis was inves- tigated. Tissues obtained from human tumors were divided into thin lamellae and placed in Eagle's basal medium containing calf serum. Double label-

ing was obtained by incubating tissues in 0.5 μCi tritiated thymidine followed by incubation in 40 μCi thymidine and rinsing in neutral buffered formalin. Histological examinations were performed on all stained tissue sections. The labeling index was determined by counting the percentage of heavily-labeled nuclei among germ cells. The duration of the S phase was estimated from the ratio of heavily-labeled to weakly-labeled cells. Kinetics of cell proliferation in benign and malignant tumors of the human epidermis revealed the development of thick interwoven epithelial tracts with basal cells comprising the majority of cells present in seborrheic keratosis. The S period duration was found to be 9.2 after six hr. Labeling was found to be distributed throughout the cells with cells exhibiting inability to incorporate tritiated thymidine. Fibroepithelial tumors exhibited labeling primarily in the small buds of basophilic cells. The S phase of epithelial septa was found to be 10.3 hr. An equal distribution of labeling of basoloid cells was observed in basal cell nevus syndrome. The labeling index and S period had a longer duration in lesions as opposed to normal epidermis. Data obtained on the S period phase in basophilic cells is suggested to be an aid in the classification of malignant states that possibly evolve into basal cell carcinoma.

1721 CONCENTRATIONS OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN SOME MINERAL OILS: STUDY OF THE CORRESPONDING HAZARD. (Fre.) Thony, C. (INRS, Service de Chimie-Toxicologie, B.P. 27, 54500 Vandoeuvre-les-Nancy, France); Thony, J.; Lafontaine, M.; Limasset*, J. C. *Arch. Mal. Prof. Med. Trav. Secur. Soc.* 36(1/2):37-52; 1975.

1722 DETERMINATION OF ARSENIC IN BIOLOGICAL MATERIAL USING FLAME ATOMIC ABSORPTION SPECTROSCOPY. (Ger.) Woidich, H. (Forschungsinstitut der Ernahrungswirtschaft, A-1190 Wien XIX, Blaasstr. 29, Austria); Pfannhauser, W. *Z. Anal. Chem.* 276(1):61-66; 1975.

1723 OCCUPATIONAL AND COMMUNITY ASBESTOS EXPOSURE FROM WALLBOARD FINISHING COMPOUNDS. (Eng.) Nicholson, W. J. (Mount Sinai Sch. Medicine of the City Univ. New York, New York, N.Y.); Rohl, A.; Fischbein, S. A.; Selikoff, I. J. *Bull. N.Y. Acad. Med.* 51(10):1180-1181; 1975.

1724 FIBER RESEARCH, DEVELOPMENT AND TESTING AT ONTARIO RESEARCH FOUNDATION. (Eng.) Kuntze, R. A. (Dept. of Materials Chemistry, Ontario Res. Foundation, Sheridan Park, Mississauga, Ontario, Canada). *Asbestos* 57(1):4-7,10,12,14; 1975.

1725 INDUSTRIAL ANALYTICAL CHEMISTS AND OSHA REGULATIONS FOR VINYL CHLORIDE. (Eng.) Levine, S. P. (Stauffer Chemical Co., Eastern Res. Labs, Dobbs Ferry, N.Y. 10522); Hebel, K. G.; Bolton, J., Jr.; Kugel, R. E. *Anal. Chem.* 47(12):1075-1080A; 1975.

1726 SPACE-TIME CLUSTERING TESTS FOR MORE THAN TWO SAMPLES. (Eng.) Klauber, M. R. (Univ. of Utah Coll. Medicine, Salt Lake City, Utah 84132). *Biometrics* 31(3):719-726; 1975.

1727 FREQUENCY OF VESICAL NEOPLASIAS IN BOTH SEXES: A SURVEY AMONG SPANISH UROLOGISTS. (Spa.) Cifuentes Delatte, L. (Fundacion "Jimenez Diaz", Universidad Autonoma, Madrid, Spain). *Arch. Esp. Urol.* 28(4):335-350; 1975.

1728 EPIDEMIOLOGY OF CARCINOMA *IN SITU* OF THE UTERINE CERVIX. (Spa.) Villalba, R. (Instituto Oncologico "Luis Razetti", Caracas, Venezuela); Muller, G.; Munoz, E.; Martinez, P. *Rev. Obstet. Ginecol. Venez.* 34(2):181-188; 1974.

1729 OCCURRENCE OF NERVOUS-TISSUE TUMORS IN CATTLE, HORSES, CATS AND DOGS. (Eng.) Hayes, H. M., Jr. (Natl. Cancer Inst., Bethesda, Md.); Priester, W. A.; Pendergrass, T. W. *Int. J. Cancer* 15(1):39-47; 1975.

1730 INTESTINAL POLYPS IN THE NIGERIAN AFRICAN. (Eng.) Williams, A. O. (Dept. of Pathology, Univ. of Ibadan, Ibadan, Nigeria); Prince, D. L. *J. Clin. Pathol.* 28(5):367-371; 1975.

1731 PREVALENCE OF CARDIAC TUMOURS AT AUTOPSY IN IBADAN. (Eng.) Abioye, A. A. (Univ. Coll. Hosp., Ibadan, Nigeria); Maolomo, I. M. *Trop. Geogr. Med.* 27(1):25-30; 1975.

1732 CIGARETTE SMOKING, TAR CONTENT, AND DEATH-RATES FROM LUNG CANCER IN AUSTRALIAN MEN. (Eng.) Gray, N. (Anti-Cancer Counc. Victoria, East Melbourne, Australia); Hill, D. *Lancet* 1(7918):1252-1253; 1975.

1733 MORTALITY PATTERNS AMONG WORKERS EXPOSED TO CHLOROMETHYL ETHERS -- A PRELIMINARY REPORT. (Eng.) Albert, R. E. (New York Univ. Medical Center, New York, N.Y. 10016); Pasternack, B. S.; Shore, R. E.; Lippmann, M.; Nelson, N.; Ferris, B. *Environ. Health Perspect.* 11:209-214; 1975.

See also:

* (Rev): 1207, 1208, 1210, 1224, 1225, 1233, 1241, 1250, 1251, 1252, 1253, 1254, 1255, 1256
* (Chem): 1261, 1262, 1313, 1314
* (Phys): 1381
* (Path): 1601, 1629

1734 INDUCTION OF SQUAMOUS METAPLASIA (VITAMIN A DEFICIENCY) AND HYPERSECRETORY ACTIVITY IN RACHEAL ORGAN CULTURES. (Eng.) Marchok, A. C. (Carcinogenesis Program, Biology Div., Oak Ridge Natl. ab., Oak Ridge, Tenn. 37830); Cone, M. V.; Nette-sheim, P. *Lab. Invest.* 33(4):451-460; 1975.

at tracheas were maintained in organ culture with arious nutritional environments. Tracheas were ob-ained from 10- to 12-wk-old SPF inbred Fischer-344 emale rats. The level of medium (0.8-0.9 ml) in the rgan culture dishes was adjusted so that only the nderside of the explant was submerged. The types of edia used were: Waymouth's MB 752/1 (vitamin A-ree) plus 50 U penicillin and 50 μg streptomycin, or his medium supplemented with either 10% horse serum r 10% fetal calf serum. Vitamin A was added to the edia to give concentrations from 0.2-20 μg in a final oncentration of 0.1% ethanol. After selected periods f culture, the morphology of the explants was stud-ed. DNA synthesis was measured by pulse labeling or 45 min with ^{3}H-thymidine and counting in a liquid cintillation spectrometer. In Waymouth's 752/1 de-ined, vitamin-A free medium, the mucociliary epithe-ium changed to a highly active keratinizing squamous pithelium at the site of the original mucosa. Epi-helial cells that migrated from the original mucosa nd covered the underside of the tracheal explants de-eloped into keratinizing squamous epithelium within few days. These transitions were accompanied by igh rates of DNA synthesis. Addition of 10% horse erum to the Waymouth's medium completely inhibited quamous metaplasia; the actively secreting muco-iliary epithelium of the original mucosa slowly be-ame quiescent, and the undergrowth epithelium usually emianed undifferentiated. These explants maintained lower level of DNA synthesis. When vitamin A (all rans retinol) was added to the serum-supplemented edium, 0.2-2.0 μg/ml induced hypersecretory activity n the original mucosa. Addition of 2.0 μg/ml also timulated cellular hyperplasia and mitosis. A vita-in A concentration of 20 μg/ml was toxic. It is oncluded that a spectrum of epithelial cell types ormally found in the tracheal mucosa *in vivo* can be nduced and maintained in tracheal organ cultures *in tro* by utilizing the appropriate nutritional envi-onment.

'35 USE OF CELL SEPARATION AND SHORT-TERM CUL-TURE TECHNIQUES TO STUDY ERYTHROID CELL VELOPMENT. (Eng.) Glass, J. (Dept. Medicine, Beth rael Hosp. and Harvard Medical Sch., Boston, Mass. 215); Lavidor, L. M.; Robinson, S. H. *Blood* (5):705-711; 1975.

veral techniques of cell separation were combined isolate murine erythroid cells at different stages maturation in order to develop a model system for udies of erythroid cell development. Hemolytic ane-a was induced in CD-1 virgin female mice with ip jections of phenylhydrazine (30 mg/kg) on days 0, and 3. Cell populations highly enriched for the fferent stages of erythroid cell maturation were tained by harvesting spleens on day 4. Immunologic molysis using antiserum prepared in rabbits against use adult RBC and guinea pig complement eliminated the more mature RBC. The residual nucleated erythroid cells were separated by velocity sedimentation. The resulting cell fractions were studied both directly and after overnight incubation in the presence or absence of human urinary erythropoietin (0.2 U/ml). Four hundred cells were counted per point. In short-term culture, erythropoietin stimulated proliferation of pronormoblasts and basophilic normoblasts, but not cells at later stages of differentiation. Erythro-poietin also appeared to recruit increased numbers of pronormoblasts. In this system, erythroid cell dif-ferentiation proceeded in the absence of erythro-poietin, but without proliferation of these early erythroid cells. These techniques provide a model system for the study of erythroid cells at different stages of maturation isolated from a uniform source at one point in time. The morphologic observations indicate that erythropoietin stimulates erythroid cell proliferation at several early stages of the maturation pathway.

1736 DJUNGARIAN HAMSTER - A SUITABLE TOOL FOR CANCER RESEARCH AND CYTOGENETIC STUDIES. (Eng.) Pogosianz, H. E. (Inst. Experimental Clinical Oncology, Acad. Medical Sci., Kashirskoje Shousse 6, 115478 Moscow M-478, USSR). *J. Natl. Cancer Inst.* 54(3):659-664; 1975.

The Djungarian hamster, bred under usual laboratory conditions, developed different spontaneous tumors, most often mammary cancers and squamous-cell skin carcinomas. Among 165 tumors in 130 animals there were 72 mammary cancers, 33 skin carcinomas, 23 primary pulmonary cancers (22 in females and one in a male), 22 tumors of other sites (liver, uterus, ovary, and reticulum tissue), and 15 benign tumors (liver and lung adenomas and skin papillomas). Two permanent strains of transplantable mammary tumors have been established in noninbred animals, indicating that the Djungarian hamster resembles the Syrian hamster in weakness of histocompatibili-ty genes. The hamsters were susceptible to the carcinogenic action of 7,12-dimethylbenz[*a*]anthra-cene and 3-methylcholanthrene, a single 2 mg injec-tion of each carcinogen inducing tumors in 67% of 54 animals and 43% of 54, resp. Among nitroso com-pounds, methylnitrosourea (MNU) was more effective in inducing tumors than diethylnitrosamine (DEN) and dimethylnitrosamine (DMN); tumors appeared in 30 of 40 animals given repeated s.c. injections of 2.5-6.2 mg MNU. The hamsters appeared resistant to urethan (1 mg/g). Of the oncogenic viruses tes-ted, the hamsters were susceptible to all variants of Rous sarcoma virus, especially the highly onco-genic variant of the Carr-Zilber strain (100% tumor incidence), and to simian adenovirus-7*(38-83%). They were less susceptible to adenovirus-12 (22% tumor incidence) and completely resistant to simian virus 40. Polymorphism was not observed in the experimental hamster, although studies of meiosis showed some peculiarities in chromosome morphology and behavior. The chromosome-breaking effect of γ-rays, MNU, and oncogenic viruses was demonstrated in primary cell cultures. DEN, DMN, urethan, and hydroxylamine exhibited a weak chromosome-breaking effect. Karyotype abnormalities were generally not

seen in primary or induced neoplasms. The experimental data indicate that the Djungarian hamster is a useful tool for cancer research and cytogenetic studies.

1737 REPEATED CLONING FROM UNTREATED DIPLOID XC-CELLS OF HYPOTETRAPLOID XC-CELLS WITH ALTERED MORPHOLOGY, *IN VIVO* GROWTH, ELECTRIC MOBILITY AND CYCLIC AMP CONTENT. (Eng.) Ebbesen, P. (Inst. Med. Microbiol., Univ. Copenhagen, Denmark); Hesse, J.; Van Den Bergh, T.; Capito, K.; Visfeldt, J. *Eur. J. Cancer* 11(2):93-96; 1975.

Three hypotetraploid E2B4 XC-cell cultures, established by double-cloning of ordinary diploid XC-cell cultures, show fewer syncytial plaques than ordinary XC-cells when exposed to murine leukemia virus-infected fibroblasts. Infection of the di- and tetraploid cell lines with 10^6 XC-units of Rauscher virus caused no alteration of karyotype, and the XC-test for virus inducing syncytia revealed no virus in extracts of the XC-cells. The functional difference between the cell lines is thus not a result of infection after cloning, but seems related to the difference in karyotype. Growth of transplanted tumor cells, as tested by i.p. injection of 10-10^6 cells into C3H mice and Wistar rats, succeeded only in newborn rats and only at the level of 10^6 cells. Electrophoretic anodic mobility of the hypotetraploid cells was slightly higher and cyclic AMP content as determined by protein-binding radioassay, was lower than in diploid XC-cells. Progressive *in vivo* growth of the tetraploid cells may therefore, be related to the higher negative outer charge. Both the lack of cell fusion and the low AMP content of the hypotetraploid cells seem connected with a decrease in cell adhesiveness.

1738 CYCLIC AMP MODULATES MICROVILLUS FORMATION AND AGGLUTINABILITY IN TRANSFORMED AND NORMAL MOUSE FIBROBLASTS. (Eng.) Willingham, M. C. (Natl. Cancer Inst., Bethesda, Md.); Pastan, I. *Proc. Natl. Acad. Sci. U.S.A.* 72(4):1263-1267; 1975.

A novel utilization of dark field microscopy for observing the effects of dibutyryl adenosine 3':5'-monophosphate (Bt_2cAMP) and concanavalin A (Con A) on surface microvilli of living cultured cells is described. Cells grown on coverslips were viewed either under phase contrast microscopy or dark field visible light microscopy; examination of cells during agglutination by Con A was carried out with the aid of electron microscopy and immunofluorescence. Treatment with Bt_2cAMP for 24 hr caused transformed L929 cells to assume a spindly, extended shape, accompanied by regression of the numerous microvilli. Pretreatment of the cells for only 15 min with the phosphodiesterase inhibitor methylisobutylxanthine or the adenylate cyclase activator prostaglandin E_1 caused a similar regression of microvilli. Removal of Bt_2cAMP caused a reappearance of the microvilli. By contrast, cells of the untransformed 3T3-4 line revealed few microvilli under normal culture conditions. A study of the highly agglutinable transformed L929 and MNRK lines of cells revealed that the presence of Con A had no effect on the presence of microvilli. The evidence indicates that transformed cells are more agglutinable than normal cells due to the presence of large numbers of microvilli on their surfaces. It is suggested that the presence of microvilli in transformed cells is due to their low levels of cyclic AMP and that the ability of cAMP to decrease agglutinability is mediated through its ability to cause the regression of microvilli.

1739 URIDINE 3',5'-MONOPHOSPHATE (CYCLIC UMP). I. ISOLATION FROM RAT LIVER EXTRACTS. (Eng.) Bloch, A. (Dept. Experimental Therapeutics, Roswell Park Memorial Inst., Buffalo, N. Y. 14263). *Biochem. Biophys. Res. Commun.* 64(1):210-218; 1975.

During the chromatographic separation of cyclic cytidine monophosphate (CMP) from cold acid and hot water extracts of Sprague-Dawley rat liver, a second UV-absorbing material was isolated and identified as 3,5'-cyclic uridine monophosphate (UMP) by comparison with the authentic compound. Both compounds had the same Rf values in nine solvent systems, similar electrophoretic mobility in three buffers, the same UV spectra, and the same nuclear magnetic resonance spectra. Both compounds were also resistant to cleavage of the phosphodiester bond by RNase and yielded the same product on 3'-nucleotidase treatment following acid hydrolysis. In addition, authentic cyclic UMP and the isolated compound had the same effect on the growth of leukemia L-1210 cells *in vitro*. When L-1210 cells were grown to stationary phase and cooled at 4° for 1 hr, growth in the presence of fresh medium resumed only after a 2-hr lag. Addition of 1×10^{-5} authentic or isolated cyclic UMP prolonged this lag period by approximately 1.5 hr. That cyclic UMP, like cyclic AMP and cyclic GMP, delays the onset of growth of staged L-1210 cells implies that it also participates in the regulation of the cell cycle.

1740 STUDIES ON THE UPTAKE OF 2-DEOXY-D-GLUCOSE IN NORMAL AND MALIGNANT RAT EPITHELIAL LIVER CELLS IN CULTURE. (Eng.) Siddiqi, M. (Christie Hosp., Manchester, England); Iype, P. T. *Int. J. Cancer* 15(5):773-780; 1975.

Membrane permeability changes during malignant transformation of rat liver epithelial cells were investigated using 2-deoxyglucose. The possible effects of cell division and of density-dependent growth inhibition on membrane properties were examined, as were the kinetics of 2-deoxyglucose transport in normal, chemically transformed rat liver cells and hepatoma cells. Three control cell lines were used; two (RL14 and RL16) were derived from adult Wistar rat liver and the third (NRL6) from newborn rat liver. The malignant cell lines HL5 and D203 were derived from two primary hepatomas induced *in vivo* by oral administration of 4-dimethylaminoazobenzene. The cell lines C23, C35 and C36 were derived from RL14 control cells which had been treated with Aflatoxin B_1. Two other cell lines, C16 and C53, were derived from RL16 cells treated with 4-dimethylaminoazobenzene and *N*-nitrosourea, respectively. Cells at subconfluent and con-

uent stages were incubated for 20 min at 37 C with -labeled and unlabeled 2-deoxyglucose. The hepa-ma cells possessed a higher permeability to 2-oxyglucose at all stages of culture. However, a crease in the uptake of 2-deoxyglucose at conflu-cy was observed in cells that exhibited density-pendent growth inhibition, whether normal or ma-gnant. The normal cells in mitosis showed an in-eased permeability to the sugar, whereas no such ange was observed in the hepatoma cells. The ki-tic studies of 2-deoxyglucose transport in normal d transformed rat liver showed a positive corre-ation between the increase in V_{max} and the trans-rmed state of the cells, whether they were trans-rmed *in vitro* or *in vivo*. No changes in the ap-rent K_m were found, indicating that there are no alitative changes in the transport sites. The re-lts suggest that an increase in the number of sites volved in glucose transport is a characteristic of emically transformed rat liver epithelial cells.

741 REQUIREMENT OF BROMODEOXYURIDINE FOR THE MAINTENANCE OF "TRANSFORMED" CHARACTERIS-ICS IN BROMODEOXYURIDINE DEPENDENT CELLS. (Eng.) rn, D. (Children's Hosp. Medical Center, Boston, ass. 02115); Davidson, R. L. *J. Cell. Physiol.* 85 2):251-260; 1975.

he requirement of bromodeoxyuridine for the main-enance of transformed characteristics in a bromo-eoxyuridine-dependent cell line, B4, was investi-ated. This line, isolated from a highly malignant, oncontact-inhibited Syrian hamster melanoma line, equires bromodeoxyuridine for optimal growth and aintenance of the noncontacted-inhibited state. NA synthesis in B4 cells in modified Eagle's medium upplemented with 10% fetal calf serum (E medium) as studied by autoradiography. With a 24-hr label-ng time, the percent of nuclei which incorporate H-thymidine was approximately 10 times lower in con-luent cultures than in subconfluent cultures. The ells were renewed with E medium three days prior to he additions of ^{3}H-thymidine. If confluent cultures ere renewed with E medium at the time the label was dded, there was two- to six-fold increase in the ercent of cells which incorporated ^{3}H-thymidine over he levels observed when the cultures were renewed hree days prior to labeling. The addition of 10^{-3} M ibutyryl cyclic AMP (dbcAMP) to B4 cells in E medium ontaining 0.1 mM bromodeoxyuridine did not cause B4 ells to become contact inhibited, having only a very light effect on saturation density. Although dbc MP caused no change in cell morphology, it did re-uce the growth rate of the cells during the first even days to approximately that of B4 cells in E me-ium. There were no significant differences in ag-lutinability by concanavalin A between B4 cells grow-ing in the presence or absence of bromodeoxyuridine, whether the cells were analyzed in confluent or sub-confluent cultures. In addition to being contact in-hibited, B4 cells grown in the absence of bromodeoxy-uridine had a higher serum requirement, grew less well in soft agar, and were less agglutinable by wheat germ agglutinin than B4 cells grown in the presence of bro-modeoxyuridine. The results suggest that the express-ion of several but not all characteristics associated with malignant transformation can be altered in the bromodeoxyuridine-dependent cells simply by growing the cells in the presence or absence of bromodeoxy-uridine.

1742 CIRCULAR DICHROISM SPECTRA AND ETHIDIUM BROMIDE BINDING OF 5-DEOXYBROMOURIDINE-SUBSTITUTED CHROMATIN. (Eng.) Nicolini, C. (Temple Univ. Health Sci. Cent., Philadelphia, Pa.); Baserga, R. *Biochem. Biophys. Res. Commun.* 64(1):189-195; 1975.

Changes in the circular dichroism spectra of 5-deoxy-bromouridine (brUdRib)-substituted chromatin were measured in mouse 3T6 fibroblast cells. The cells were grown in a medium containing 7.2, 36, or 72 μM brUdRib, giving respectively, 4.9, 14, and 20% replace-ment of thymidine moieties by brUdRib. Ethidium bro-mide binding was measured by a circular dichroism method that assumes that only intercalated dye mole-cules acquire optical activity, while weakly bound dye molecules are not optically active. A spectro-polarimetric method could thus be used to give the number of chromatin primary binding sites for ethidi-um bromide. Changes in the circular dichroism spectra between 250-300 nm of brUdRib-substituent chromatin were dependent on the extent of replacement of the thymidine moieties. With 14 and 20% replacement there was a blue shift and a marked increase in positive el-lipticity; with 4.9% replacement, little effect was noticed. Between 200-255 nm, the circular dichroism spectra of chromatin were the same as that of control chromatin, regardless of the brUdRib concentration used. BrUdRib-substitution thus does not affect the number of ethidium bromide primary binding sites of chromatin.

1743 MITOGENIC EFFECT OF FIBROBLAST GROWTH FAC-TOR ON EARLY PASSAGE CULTURES OF HUMAN AND MURINE FIBROBLASTS. (Eng.) Gospodarowicz, D. (Salk Inst. for Biological Studies, San Diego, Calif. 92112); Moran, J. S. *J. Cell Biol.* 66(2):451-457; 1975.

The mitogenic effect of fibroblast growth factor was tested on diploid, early-passage cells from human and murine sources to ascertain its role in the con-trol of cell division. Diploid human foreskin fibroblasts and mouse fibroblasts were cultured in Dulbecco's medium. Cells were plated in 5 ml medium with 2.5% (human cells) or 10% (murine cells) calf serum for growth rate determination. After one day (human cells) or three days (murine cells), the media were replaced with media containing 0.2% (human cells) or 0.4% (murine cells) calf serum. After two days (human cells) or one day (murine cells) in low serum, fibroblast growth factor and insulin were added in 25-100 μl of a 0.5% solution of crystalline bovine serum albumin in medium. Dexamethasone was added in 25-50 μl of absolute ethanol. Fibroblast growth fac-tor, insulin, and dexamethasone were added daily; serum was added only once. Cells were counted after trypsinization. The quantitative assay of [^{3}H]thy-midine incorporation into acid-insoluble material showed that fibroblast growth factor at low concen-trations (10^{-9} M) was more effective than additional serum for provoking the initiation of DNA synthesis

in human foreskin fibroblasts or mouse fibroblasts maintained in 5 or 10% serum, respectively. The growth of the human fibroblasts was twice as fast in the presence of fibroblast plus 10% calf serum as it was in the presence of 10% calf serum or 20% fetal calf serum alone. The addition of fibroblast growth factor to primary cultures of mouse fibroblasts in 0.4% serum resulted in a 2-fold increase in cell number compared to controls. In contrast to previously published results with 3T3 cells, neither insulin nor a glucocorticoid potentiated the effects of fibroblast growth factor on either human or mouse cells. The results indicate that fibroblast growth factor is mitogenic not only for established heteroploid cell lines, but also for early passage cultures of diploid cells. The fibroblast growth factor lacks species specificity, because it acts on human as well as mouse fibroblasts.

1744 MLC STIMULATORY CAPACITY AND PRODUCTION OF A BLASTOGENIC FACTOR IN PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA AND HODGKIN'S DISEASE. (Eng.) Kasakura, S. (R. Victoria Hosp., Montreal, Canada). *Blood* 45(6):823-832; 1975.

The stimulatory capacity of chronic lymphatic leukemia (CLL) and Hodgkin's disease (HD) lymphocytes in the one-way mixed lymphocyte reaction (MLR) was evaluated. In patients with these diseases, the question of whether a correlation exists between the degree of stimulatory capacity and production of a blastogenic factor was investigated. Ten untreated patients with CLL and ten untreated patients with Hodgkin's disease were studied. Stimulatory capacity of CLL lymphocytes was intact or increased, while their responding capacity was markedly depressed. In the patients with advanced Hodgkin's disease, both stimulating and responding capacities were impaired. In these patients, a correlation existed between the degree of stimulatory capacity and production of a blastogenic factor. This observation would raise the possibility that the production of a blastogenic factor may be involved in the stimulation of responding lymphocytes in mixed cultures. These results, which show a dichotomy between MLC stimulatory and responding capacities of CLL lymphocytes, may suggest that different factors are involved in stimulation and response.

1745 CONSECUTIVE CYTOCHEMICAL STAINING FOR THE ANALYSIS OF THE BLASTIC POPULATION IN THE ACUTE PHASE OF CHRONIC MYELOID LEUKEMIA. (Eng.) Castoldi, G. L. (Clinica Medica Generale, Universita Ferrara, 44100 Ferrara, Italy); Grusovin, G. D.; Scapoli, G. L. *Biomedicine* 23(1):12-16; 1975.

The blastic population in the acute phase of chronic myeloid leukemia (CML) was analyzed in 14 patients using consecutive cytochemical staining. Smears from the peripheral blood of these patients were submitted to a series of cytochemical reactions [periodic acid-Schiff (PAS), Sudan black B, peroxidase, acid phosphatase, nonspecific esterases, β-aminopeptidase]. A sequence of three reactions applied consecutively on the same slide (α-naphthyl-acetate esterase + AS D-chloroacetate esterase + PAS) was useful for the detection of differently oriented blast cells. A common feature of these cases was the presence of cells with the appearance of diploid megakaryoblasts and of myeloid cells with differentiation toward monocytoid properties. Megakaryoblasts were characterized in tri-stained preparations by a strong α-naphthyl-acetate esterase reaction and sometimes hallmarked by a PAS positive surrounding material possibly related to platelet differentiation. Monocytoid elements were characterized by a usually strong α-naphthyl-acetate esterase positive reaction and by a fine granular PAS positivity of the cytoplasm. Basophilic precursors were often present and characterized by coarse PAS precipitates on the border of the cytoplasm, and by the presence of a variable degree of positivity to both the α-naphthyl-acetate and the AS D-chloroacetate esterase reactions. Lymphoblastic elements were not detected. β-glucuronidase activity of the type commonly observed in lymphoblasts was present in only a very small percent of the cells. These results suggest that blastic crisis in CML is associated with a complex population of blast cells, at least from the cytochemical point of view. The data are in agreement with the concept that the blast cells in the acute phase of CML arise from a common precursor, involving all the myeloid series, and support the idea that the CML in the acute stage reflects a basic maturation defect of the stem cells.

1746 HERITABILITY OF THE PHYTOHEMAGGLUTININ RESPONSIVENESS OF LYMPHOCYTES AND ITS RELATIONSHIP TO LEUKEMOGENESIS. (Eng.) Heiniger, H. J. (Jackson Lab., Bar Harbor, Maine); Taylor, B. A.; Hards, E. J.; Meier, H. *Cancer Res.* 35(3):825-831; 1975.

The strain distribution pattern of stimulation of peripheral mouse lymphocytes by phytohemmagglutinin (PHA) was established by tests with 59 inbred, F_1 hybrid, and congenic-resistant (CR) strains. Whole blood cultures from 5-10 males of each strain were incubated at 37 C for 36 hr with 250 μg/ml PHA, followed by incubation for 16 hr with tritiated thymidine [(^{3}H)TdR]. Monitoring of radioactivity was performed using a liquid scintillation counter. When 11 H-2 CR strains were tested for PHA response in relation to histo-compatibility, no linkage between H-2 gene and genes governing the PHA was evident. The DBA family showed a low response to PHA, whereas the C57 family showed a high response. Strain PL/J was the highest responder. The response of lymphocytes to the PHA is estimated to be controlled by more than two but less than five genes. Genes conferred by DBA strains were dominant over the allele(s) from C57. In hybrids other than those with DBA strains, the C57 alleles were dominant, conferring a high response. High leukemia strains AKR/J, C58/J, and PL/J were all high PHA responders, and no strain with a high spontaneous incidence of leukemia was found among the low responders. No correlation exists between expression of the type-C RNA genome and PHA response. The heritability of the PHA response is 75%-80%. The continuous distribution of PHA responsiveness exhibited by the 59 strains indicates multifactorial inheritance of the trait.

1747 CHANGES IN RNA IN RELATION TO GROWTH OF THE FIBROBLAST. III. POSTTRANSCRIPTIONAL REGULATION OF mRNA FORMATION IN RESTING AND GROWING CELLS. (Eng.) Johnson, L. F. (Dept. Biol., Massachusetts Inst. Technol., Cambridge); Williams, J. G.; Abelson, H. T.; Green, H.; Penman. S. *Cell* 4(1):69-75; 1975.

The proportion of heterogeneous nuclear RNA (hnRNA) processed into cytoplasmic messenger RNA (mRNA) in resting and growing 3T3 and 3T6 cells was investigated by measuring the efficiency of transfer of nuclear poly(A) to the cytoplasm. When nuclear poly(A) was labeled with ^{3}H-adenosine, either continuously with precursor or pulse labeled, and then chased with cordycepin, growing cells transferred approximately twice as much of the poly(A) from the nucleus to the cytoplasm as did resting cells. When cells underwent a serum-induced transition from the resting to growing state, the efficiency of poly(A) transfer was increased to that characteristic of growing cells by three hours after the addition of the serum. The proportion of hnRNA which was polyadenylated and the total nuclear poly(A) content were the same in resting and growing cells. The results suggest that the principal means by which the cell regulates its poly(A)+ mRNA content in the growing and resting states is by regulating the efficiency with which nuclear poly(A)+ RNA is converted to cytoplasmic mRNA.

1748 DOUBLE STRANDED RIBONUCLEIC ACID IN HUMAN LEUKEMIC BLAST CELLS. (Eng.) Torelli, U. (Inst. Med. Pathol., Univ. Modena, Italy); Torelli, G.; Cadossi, R. *Eur. J. Cancer* 11(2):117-121; 1975.

Double-stranded RNA (dsRNA) in many types of cells is bound to polyadenylate [poly(A)] sequences. The occurrence in leukemic blast cells of a large proportion of poly(A)-bound nuclear RNA led to this study of RNA extracted from leukemic blast cells of four patients with acute myeloid leukemia, (2 x 10^6 cells/ml cultured at 37 C) cells were labeled with (^{3}H-5)-uridine and (^{3}H)-adenosine (10 μCi/ml) and incubated up to nine hr. dsRNA was isolated by selective nuclease digestion (DNase, 20 μg/ml; pancreatic RNase, 30 μg/ml; Tl RNase, 20 U/ml; subtilisin, 50 μg) and cellulose chromatography. DNA, transfer RNA and single-stranded RNA eluted in 35% and 15% ethanol and buffer, while dsRNA eluted in buffer alone. From 5% to 15% of (^{3}H)-uridine-labeled RNA remained TCA-precipitable after nuclease digestion. Up to 28% of (^{3}H)-adenosine-treated RNA was RNase resistant. The proportion of dsRNA bound to poly(A) sequences (23 to 27%) was evaluated with two samples by measuring labeled dsRNA bound to glass fiber filters on which poly(U) had been immobilized. Two portions of fractionated cell suspensions showed that 83% and 91% of the total dsRNA in each came from the nucleus. When incubation was extended from 3 hr to 9 hr, a significant increase of dsRNA occurred *in vitro*. The findings indicated that dsRNA is synthesized in leukemic blast cells.

1749 DIFFERENCES IN THE CYTOPLASMIC DISTRIBUTION OF NEWLY SYNTHESIZED POLY(A) IN SERUM-STIMULATED AND RESTING CULTURES OF BALB/c 3T3 CELLS. (Eng.) Bandman E. (Dep. Mol. Biol., Univ. California, Berkely); Gurney, T., Jr. *Exp. Cell Res.* 90(1):159-168; 1975.

Since only messenger RNA has been shown to contain polyadenylate regions, polyadenylate content was used as a marker for messenger RNA in polysomes and in the subribosomal fraction. Recloned BALB 3T3 cells were grown at 37 C. Serum stimulation was performed by replacing the medium from resting cells with 20% calf serum. Recloned SV40-transformed BALB 3T3 cells were grown in the same way except that the medium contained 2% calf serum. Cell cultures were incubated for 90 min at 37 C with 2,8-^{3}H adenosine and the messenger RNA was isolated and quantitated by virtue of its association with the newly-synthesized radioactive polyadenylate. The rate of accumulation of cytoplasmic polyadenylate rose slowly after serum stimulation, reaching a value of 1.8 times that of resting cultures at 12 hr after stimulation, which was also the time of onset of DNA synthesis. A change in the cytoplasmic distribution of newly-synthesized polyadenylate occurred more rapidly than the change in the rate of its synthesis. Resting cultures contained 37% of newly-synthesized cytoplasmic polyadenylate-containing RNA large enough to be messenger RNA in the post-ribosomal cell fraction, whereas virtually all of this material was found in polyribosomes at 3, 6, and 12 hr after stimulation and in transformed cultures. The relatively infrequently translated messenger RNA of resting cultures was shown to be functional by cycloheximide treatment. All BALB/c 3T3 cultures, resting or stimulated, contained about 20% of the newly synthesized cytoplasmic polyadenylate as molecules of 4-6 Svedbergs in size, presumably too small to be messenger RNA. It is concluded that serum stimulation of density-inhibited cultures results in a more efficient use of the protein-synthesizing ability of the cell, and that the change in efficiency precedes increases in the numbers of ribosomes and messenger RNA molecules.

1750 INCREASED FREQUENCY OF INITIATION OF RNA SYNTHESIS DUE TO A PROTEIN FACTOR FROM CHICKEN MYELOBLASTOSIS NUCLEI. (Eng.) Chuang, R. Y. (Duke Univ. Medical Center, Durham, N.C. 27710); Chuang, L. F. *Proc. Natl. Acad. Sci. USA* 72(8):2935-2939; 1975.

The mechanism of the effect of an RNA polymerase II (RNA nucleotidyltransferase II) stimulation factor isolated from the nuclei of chicken myeloblastosis cells was studied. The stimulation requires the presence of all four nucleoside triphosphates and depends upon an exogenous DNA template. In the absence of the factor, RNA synthesis ceases after 20-30 min, but in the presence of the factor, synthesis continues up to 60-80 min. Addition of the factor at 35 min after incubation causes resumption of RNA synthesis. The factor greatly stimulates the activity of RNA polymerase II at low enzyme concentrations. The RNA polymerase activity is more sensitive to α-amanitin inhibition when the factor is present. Experiments of [γ-^{32}P]-ATP incorporation reveal that the factor provides for an increased frequency of initiation of RNA chains, both of the primary initiation events and re-initiation after previous ones were completed. A slightly higher rate of RNA chain growth was also observed

with this factor, but the ultimate size of RNA synthesized was not affected, as determined by formaldehyde/sucrose gradient centrifugation. These data suggest that the factor functions at the initiation stages of the RNA polymerase reaction. This system facilitates the study of RNA synthesis where prolonged transcription is required and where transcription is dependent on initiation.

1751 INITIATION OF DNA SYNTHESIS IN MOUSE 3T3 FIBROBLASTS IN RESPONSE TO A SPECIFIC FACTOR. (Eng.) Wolf, L. (Milton S. Hershey Medical Center, Hershey, Pa. 17033); Kohler, N.; Roehm, C.; Lipton, A. *Exp. Cell Res.* 92(1):63-69; 1975.

Serum and plasma from female Sprague-Dawley rats were assayed for their ability to promote multiplication and initiate DNA synthesis in mouse 3T3 fibroblast cells. The DNA-initiating activity in rat serum and plasma became undetectable at the same concentrations when serial dilutions were used. However, the factor that promotes 3T3 replication was much greater in rat serum than in plasma over the same dose range. Depleted medium obtained from confluent 3T3 cells contained low amounts of 3T3 multiplication-stimulation activity, while medium harvested from growing 3T3 or simian virus 40-transformed 3T3 cells was partially depleted of 3T3 DNA-initiating activity. The DNA-initiating activity in rat serum and the serum multiplication factor were stable from pH 2 to pH 11. Serum DNA-initiating and multiplication-stimulating activities did not differ in their response to treatment with 3 M NaCl, 4 M guanidine hydrochloride, or 0.05% sodium dodecyl sulfate. Both activities retained their stability during repeated freeze thaw, and both were present in human urine. A plasma fraction that promotes DNA-initiation of 3T3 cells and is not active in the multiplication assay was obtained by rechromatography of peaks obtained on Sephadex G-100 at pH 2. The activity of this fraction was destroyed by pepsin treatment. Attempts to separate serum DNA initiating activity from multiplication-stimulating activity were unsuccessful. It is concluded that the DNA-initiating factor separated from rat plasma enables 3T3 cells to move from a resting state through the G1-2 barrier.

1752 PLASMINOGEN ACTIVATORS IN OVARIAN TUMOURS. (Eng.) Svanberg, L. (Coagulation Lab., Univ. Lund, Sweden); Linell, F.; Pandolfi, M.; Astedt, B. *Acta Pathol. Microbiol. Scand.* [*A*] 83(2):193-198; 1975.

Biopsy specimens of 25 fresh ovarian tumors and 14 normal ovaries were obtained at laparotomy of non-irradiated patients. The tumors were histochemically examined for their local fibrinolytic activity, and simultaneous fibrin/fibrinogen degradation products (FDP) were determined in the serum. The fibrinolytic activity in malignant and benign ovarian neoplasms, as well as in normal ovaries, was found to originate from vessels. A very close correlation was demonstrated between the fibrinolytic activity and the vascularity of the sections but there was no correlation with malignancy. FDP were found in the serum in 13 of 14 patients with malignant tumors, but in none with benign tumors. The difference in occurrence of FDP in patients with malignant and benign tumors might be due to the invasive growth of the former with the entrance of thromboplastic substances, fibrinolytic activators or locally formed FDP into the bloodstream.

1753 EFFECT OF REDUCED TEMPERATURES ON PROTEIN SYNTHESIS IN MOUSE L CELLS. (Eng.) Craig, N. (Dept. Biol. Sci., Univ. Maryland Baltimore County, Catonsville, Md. 21228). *Cell* 4(4):329-335; 1975.

The rate of incorporation of leucine into protein, the rate of polypeptide elongation and termination, and the relative quantity and size of polysomes were analyzed in mouse L cells grown in suspension culture at various temperatures between 0 C and 36 C. Cells were exposed to each temperature for 20-60 min, and then concentrated 8- to 10-fold and leveled at the same temperature with ^3H- or ^{14}C-leucine. The rate of incorporation at a specific temperature was compared to the control rate in cells at 36 C to determine the relative rate of synthesis. Between 10 C and 36 C protein synthesis exhibited two different apparent activation energies (39 kcal/mole, 10-25 C; 14 kcal/mole, 25-36 C), whereas elongation and termination had only one (16 kcal/mole). Below 36 C, the polysome level and size decreased, reaching a minimum of 30% of the control 36 C values at 10 C; below 10 C the level increased again back to control values at 0 C. The polysome decline was time dependent, requiring about 5 hr to reach the equilibrium value. This decline was completely reversible within 60 min, even in the presence of 4 μg/ml actinomycin D, and even after 15 hr of incubation at the lower temperature. The results suggest that polypeptide initiation is rate limiting for protein synthesis, particularly below 25 C; above this temperature, elongation or perhaps some other process may be limiting. These results are quite different from those obtained for *E. coli* and rabbit reticulocyte protein synthesis.

1754 PRESENCE OF NEUROPHYSIN PROTEINS IN TUMORS ASSOCIATED WITH THE SYNDROME OF INAPPROPRIATE ADH SECRETION. (Eng.) Hamilton, B. P. (VA Hosp., Baltimore, Md.). *Ann. N.Y. Acad. Sci.* 248:153-156; 1975.

The presence of neurophysin proteins in tumors associated with the syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) was assessed in tumor tissue from eight patients. Tissue was obtained at autopsy and extracted for vasopressin and neurophysin proteins, seperately for three patients and pooled for the other five. Tumor tissue was also obtained and analyzed from three groups of patients in whom the diagnosis of SIADH had been excluded in life. Neurophysin was measured by radioimmunoassay, and the bioactivity of the ADH fractions was assayed in rats. Of 34 fractions assayed in seven tumor groups, four had significant levels of neurophysin and came from the three individuals with SIADH. In these tumors other sized fractions had undetectable neurophysin activity. In the tumor groups unassociated with the syndrome, all fractions assayed had less than 1 ng/mg of neurophysin. Neurophysin was undetectable in the pooled tumor group with the syndrome. In the syn-

...ome tumor specimens, ADH-like activity was demonstrated in the fractions of the molecular size of vasopressin. It is thought that vasopressin and neurophysin are produced together in tumors associated with SIADH. The evidence supports the view that ectopic hormones arise from derepression of the normal genetic information.

1755 ALTERATION OF THE FATTY ACID COMPOSITION OF EHRLICH ASCITES TUMOR CELL LIPIDS. (Eng.) Liepkalns, V. A. (Dept. Biochemistry, Univ. Iowa, Iowa City, Iowa 52242); Spector, A. A. *Biochem. Biophys. Res. Commun.* 63(4):1043-1047; 1975.

The effects of dietary fat on the fatty acid composition of Ehrlich ascites tumor cell lipids were investigated in male CBA mice. Mice were placed on one of four diets 50 days prior to inoculation of the tumor, and the cells were obtained 12 days after transplantation. The control diet was Rockland mouse laboratory chow containing 4.5% fat, made of 35% saturated, 31% monoenoic-ω9 and 30% polyenoic-ω6 fatty acids. The two test diets were standard mixtures to which were added either 16% coconut oil (87% saturated fatty acids) or sunflower oil (58% polyenoic-ω6 fatty acids). A fourth diet containing only the fat-free standard mixture was also employed. Considerable changes were produced in the fatty acid composition of the polar and neutral lipids of the Ehrlich ascites cells when the type of fat fed to the tumor bearing mouse was varied. Cells from mice fed coconut oil had much higher contents of ω9-monoenoic fatty acids and much lower amounts of the ω6- and ω3-polyenoic acids. By contrast, cells obtained from mice fed sunflower oil had higher levels of saturated and ω6-polyenoic acids and lower levels of ω9-monoenoic and ω3-polyenoic acids. Cells from animals fed the fat-deficient diet contained large amounts of ω9-monoenoic acids and very little ω6-polyenoic acids. The lipids of the tumor plasma lipoproteins and liver from the tumor-bearing mouse exhibited changes in fatty acid composition similar to those occurring in the tumor cells. Similar, but smaller changes were noted in animals fed a special diet for only 16 or 30 days. This system provides a model for investigating the relationships between lipid structure, membrane properties, and cell function in a rapidly growing tumor. The system even offers some advantages for studies of this type concerning mammalian cells in general. Ehrlich cells can be produced in extremely large amounts at only a fraction of the effort and cost required to maintain cells in long-term culture. Therefore, this sytem is especially useful for experiments requiring the preparation of large quantities of plasma membranes from a mammalian cell suspension.

1756 LIPID COMPOSITION OF PLASMA MEMBRANES FROM HUMAN LEUKEMIC LYMPHOCYTES. (Eng.) Hildebrand, J. (Service de Médecine Interne et d'Investigation Clinique de l'Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Bruxelles, Belgique); Marique, D.; Vanhouche, J. *J. Lipid Res.* 16(3):195-199; 1975.

The distribution of adenosine 5'-monophosphatase (5'-AMPase), and various lipids in whole homogenates and plasma membranes of human leukemic lymphocytes was investigated. Preparations of lymphocytes were taken from a patient with chronic lymphocytic leukemia (CLL) during a one year period. The lymphocytes were homogenized, homogenates were fractionated by centrifugation procedures, 5'-AMPase activity was assayed, total lipids were extracted, glycolipids were isolated, and total cholesterol and total phospholipids were measured. Two neutral glycolipids were found in the whole homogenates: glucosylceramide (CMH) and lactosylceramide (CDH); they were qualitatively the same in the various subcellular fractions. The greatest enrichment in measured substances were found in S1, a top fraction collected on a continuous sucrose gradient performed with the microsomal pellet, and in S2B, the plasma membrane fraction. The calculated molar ratios of cholesterol to phospholipids were 0.33 and 0.68 for S1 and S2B, resp. 5'-AMPase activity was enriched 69.5 ± 27.3 times in the plasma membrane fraction, while the CDH concentration increased 34.4 ± 14.1 times. Phospholipids in the whole leukemic lymphocytes and in plasma membrane preparations were qualitatively the same. However, the pattern in lymphocyte plasma membranes, compared to that in the whole homogenate, was characterized by a decrease of phosphatidylcholine and an increase of sphingomyelin. The highest enrichment in CDH was associated with the highest enrichment in 5'-AMPase and the highest molar ratio of cholesterol:phospholipids. The results agree with previous reports that glycolipids are located primarily but not exclusively on the cell surface, the subcellular localization subject to variation according to cell type and glycolipid type.

1757 ENZYME ACTIVITIES AT THE SURFACE OF INTACT EHRLICH TUMOR CELLS WITH ALBUMIN IN THE ISOTONIC ASSAY MEDIUM. (Eng.) Wernstedt, C. O. (Biomed. Cent., Univ. Uppsala, Sweden); Agren, G. K.; Ronquist, G. *Cancer Res.* 35(6):1536-1541; 1975.

The activities of several enzymes were determined, including those that catalyze the ATP formation on the cell surface of the intact Ehrlich cell, as well as the corresponding activity of the cell homogenate representing the total activity. Membrane stabilizers, i.e. albumin and dextran, were included in the incubation media. A plasma membrane fraction from Ehrlich cells was analyzed for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and 3-phosphoglycerate kinase (PGK). GAPDH and PGK were present on the surface of intact Ehrlich tumor cells. Aldolase represented cytoplasmic enzymes not present on the external surface, provided 2.5% of bovine albumin was in the isotonic assay medium. A flux of aldolase from the cell interior to the cell exterior occurred in the absence of albumin. Therefore, any enzymatic activity monitored when keeping the Ehrlich tumor cells in the assay medium containing 2.5% albumin was considered to be primarily related to the outside of the plasma membrane. Of the total GAPDH, 0.7% was located on the outer surface of the tumor cell, while the corresponding figure for PGK was 2.7%. Of this surface-located PGK, 80% was released into the assay medium during incu-

bation, while the release of GAPDH, at the same time, was minimal. A plasma membrane preparation of Ehrlich cells, mainly consisting of vesicles, showed the presence of PGK but the absence of GAPDH. Because of the vesicular nature of the membrane preparation, it was assumed that only one side of the membrane was exposed during assay. The results indicate that of the two enzymes, PGK was the more abundant on the cell surface. Differences in abundances of PGK and GAPDH reflect binding properties, and possibly localization, within the membrane.

1758 CELL ATTACHMENT TO A SUBSTRATUM AND CELL SURFACE PROTEASES. (Eng.) Grinnell, F. (Univ. Texas Southwestern Medical Sch., Dallas, Tex. 75235). *Arch. Biochem. Biophys.* 169(2):474-482; 1975.

The attachment of baby hamster kidney (BHK-21-13s) cells to a substratum in serum-containing medium was inhibited when inhibitors of proteolytic enzymes were added to the incubation medium. The inhibitors found to be effective were L-1-tosylamide-2-phenylethyl chloromethyl ketone, *N*-α-*p*-tosyl-L-lysine choloromethyl ketone HCl, and phenylmethyl sulfonyl fluoride. Comparative studies were carried out with normal, virus-transformed and suspension-culture adapted BHK cells. The cells were treated with various concentrations of L-1-tosylamide-2-phenylethyl chloromethyl ketone and then resuspended in fresh medium and tested for their adhesiveness. The efficiency of inhibition by L-1-tosylamide-2-phenylethyl chloromethyl ketone was similar with all three cell lines. The possibility was investigated that cell attachment involves proteolytic activation by cell surface proteases of serum proteins absorbed to the substratum. It was found that trypsin or chymotrypsin treatment of substrata, coated with serum, resulted in an enhanced rate of subsequent cell attachment to the substrata. This effect did not involve bulk removal of serum from the substrata or the presence of residual trypsin in the incubations. Moreover, inhibition of cell attachment by L-1-tosylamide-2-phenylethyl chloromethyl ketone was less pronounced when cell attachment to trypsin-activated substrata was compared with attachment to unactivated substrata.

1759 CHARACTERIZATION OF NUCLEAR AND CHROMATIN POLY A-CONTAINING RNA. (Eng.) Sakamoto, S. (Baylor Coll. Medicine, Houston, Tex. 77025); Rao, M. S.; Wu, B.; Spohn, W. H.; Busch, H. *Physiol. Chem. Phys.* 7(4):309-324; 1975.

In order to isolate and analyze the poly A-containing RNA from chromatin, nuclei and cytoplasm, and to measure their relative translational capacities, Novikoff hepatoma ascites cells were labeled *in vitro* with ^{32}P-orthophosphate. Poly A-containing RNA was isolated from chromatin on nitrocellulose membrane filters after the cells were labeled for six hr. Under denaturing conditions, the poly A-containing chromatin RNA had a sedimentation coefficient of 8-18S. The presence of poly A tracts in the chromatin RNA fractions indicate that poly A addition occurs almost immediately after transcription. Poly A-containing RNA and nuclear RNA were approximately 1/5 as active as cytoplasmic poly A-containing RNA in the wheat germ translational system. Poly U-Sepharose was used for isolation of RNA for the studies of translation because this activity was very low if nitrocellulose was used. Since chromatin poly A-containing RNA is the precursor of cytoplasmic poly A-containing RNA, further processing of this RNA prior to its attachment to polysomes may account for its increase in translational capacity.

1760 PROGRESSION FROM HORMONE DEPENDENCE TO AUTONOMY IN MAMMARY TUMORS AS AN *IN VIVO* MANIFESTATION OF SEQUENTIAL CLONAL SELECTION. (Eng.) Kim, U. (Rosewell Park Memorial Inst., New York State Dept. of Health, Buffalo, N.Y. 14263); Depowski, M. J. *Cancer Res.* 35(8):2068-2077; 1975.

The natural history of breast cancer was studied by successively transplanting the earliest possible form of a rat mammary adenocarcinoma in syngeneic rats and isolating sublines of this tumor manifesting new endocrinological and other biological characteristics over a period of 15 yr. The original, fully mammotropin-dependent tumor MT-W9, gave rise to an estrogen-dependent variant, MT-W9A which grew only in normal adult female hosts and regressed promptly upon oophorectomy. This tumor produced a subline, the fully autonomous MT-W9B, which grew well in any syngeneic rats regardless of their hormonal status. The third subline derived from the autonomous tumor, MT-W9C, grew better in male than in female rats. Chromosomal analysis of these four tumors disclosed four distinct stem cell lines, but each tumor also contained small numbers of cells from other stem lines. The progression from mammotropin-dependence to androgen-responsiveness in this mammary tumor system seems to have been accomplished by shifting from one stem line to another in an orderly irreversible sequence. The progression of the tumor is accompanied by a gradual increase of cachexia-producing effects. These four distinct hormonal characteristics encompass the entire known spectrum of breast cancer in man and animals with the exception of the metastasizing property. The hormonal and cytogenetic characteristics of each stem cell line have been stable for over a decade.

1761 STUDIES ON ECTOPIC ACTH-PRODUCING TUMORS. III. AN ECTOPIC MELANOCYTE-STIMULATING HORMONE: GEL CHROMATOGRAPHIC FINDING AND TIME-LAPSE COLOR CHANGE OF FROG SKIN. (Eng.) Nakamura, M. (Shionogi Res. Lab., Shionogi Co., Ltd., Osaka, Japan); Tanaka, A.; Kawabata, T.; Yamamoto, H.; Imura, H. *Jpn. J. Clin. Oncol.* 5(1):29-32; 1975.

The melanocyte-stimulating hormone (MSH) in a metastatic liver tumor from islet cell carcinoma of the pancreas was isolated by Sephadex G-25 gel chromatography. The peak of melanocyte-stimulating activity was separable from that of adrenocorticotropic hormone activity, and was recovered in the fractions corresponding to α-MSH. However, the time course of the skin darkening response of African frogs to the tumor MSH resembled that produced by long-acting human β-MSH. These results show that the ectopic MSH was noted in the average input resistance after treatment. A change in membrane potential as it relates

a the metastatic liver tumor is a peptide differing rom human β-MSH in its chromatographic characteristics but resembling β-MSH in its biological properies.

762 BIOLOGICAL MARKERS IN BREAST CARCINOMA. I. INCIDENCE OF ABNORMALITIES OF CEA, CG, THREE POLYAMINES, AND THREE MINOR NUCLEOSIDES. Eng.) Tormey, D. C. (Natl. Cancer Inst., Bethesda, d.); Waalkes, T. P.; Ahmann, D.; Gehrke, C. W.; umwatt, R. W.; Snyder, J.; Hansen, H. *Cancer* 5(4):1095-1100; 1975.

atients with breast carcinoma were screened for bnormal plasma concentrations of carcinoembryonic ntigen (CEA), abnormal serum human chorionic gonadoropin (HCG) levels, and abnormal urinary concenrations of putrescine, spermidine, spermine, pseudoridine, N^2,N^2-dimethylguanosine, and 1-methylinoine. Four groups of patients were studied: reoperative patients without evidence of metastatic isease; patients free of disease after a radical astectomy; patients with no evidence of disease ollowing surgical excision for a first recurrence; nd those with demonstrable metastatic disease. atients in the first two groups were subdivided nto those with and those without nodal involvement. hree of nine preoperative patients had abnormal ^{2},N^2-dimethylguanosine levels and three had abnoral 1-methylinosine concentrations. Among the 18 ostoperative patients with nodal involvement, one ad an abnormal pseudouridine level, eight had an bnormal N^2,N^2-dimethylguanosine concentration, nd six had increased 1-methylinosine levels. Of he patients tested after excision of a first reurrence, pseudouridine was abnormal in 2 of 5, -methylinosine in 1 of 4, and N^2,N^2-dimethylguanoine in 4 of 4. Fifty-seven percent of 75 patients ith matastatic disease had abnormal N^2,N^2-dimethyluanosine levels; 1-methylinosine and pseudouridine evels were abnormal in 44% and 23% of these paients, resp. CEA concentrations were abnormal n 2 of 7 preoperative patients, 6 of 20 postoperaive patients with nodal involvement, and 53 of 2 patients with metastatic disease. HCG levels ere elevated in 4 of 8 preoperative patients; in of 20 postoperative patients with nodal involveent, and in 37 of 74 patients with metastases. utrescine was abnormal in 10 of 72 patients with etastatic disease; spermidine was abnormal in ight patients; and spermine was abnormal in one. he other three groups tended to have normal polyamine levels. By combining the tests for HCG, CEA, nd N^2,N^2-dimethylguanosine, it was possible to etect one or more marker abnormalities in 97% of he patients with metastatic disease and 67% of he postoperative patients with nodal involvement.

763 CELL CONTACT-DEPENDENT GANGLIOSIDE CHANGES IN MOUSE 3T3 FIBROBLASTS AND A SUPPRESSED IALIDASE ACTIVITY ON CELL CONTENT. (Eng.) ogeeswaran, G. (Sch. Public Health, Univ. Washington, eattle); Hakomori, S. *Biochemistry* 14(10):2151-156; 1975.

tudies were carried out to determine if contact-dependent enhancement of ganglioside concentration occurs in contact-sensitive mouse fibroblast 3T3 cells. The chemical quantity and incorporation of radioactivity from ^{14}C-galactose into gangliosides were studied in normal and transformed 3T3 cells at various cell population densities. Sialidase, sialyltransferase, and galactosyltransferase activities were also assayed. The chemical quantity of and the incorporation of ^{14}C radioactivity into GD1a ganglioside increased significantly at the early stage of cell contact. This response was not detectable at any stage of cell contact in 3T3 cells transformed by simian virus 40 (SV40), cells doubly transformed by polyoma virus and SV40, or spontaneously transformed cells. In membrane preparations or intact monolayers of normal 3T3 cells, sialidase activity was suppressed 50% at the 'touching' stage of cell-to-cell contact, as compared with sparse growing and 'crowded' stages. Transformed cells showed no change in sialidase activity at any stage of cell contact. The ratio of galactosyltransferase to sialyltransferase activity, measured by the ratio of ^{14}C-CMP-*N*-acetylneuraminate incorporated to that of ^{3}H-UDP-galactose incorporation into ganglioside, decreased at the early stage of cell-to-cell contact. This indicated that the change in sialidase activity in normal cells was independent of sialyltransferase activity. Additional study revealed that sialidase inhibitors such as copper acetate and *N*-phenyloxamic acid also inhibited sialyltransferase activity. Thus, the increase in sialyltransferase activity may have been due to suppressed sialidase activity. These results suggest that surface sialidase plays an important role in determining cell social activity and surface-mediated regulation of ganglioside metabolism during cell growth.

1764 FUNCTIONAL INVOLVEMENT OF SPECIFIC CARBOHYDRATE IN TERATOMA CELL ADHESION FACTOR. (Eng.) Oppenheimer, S. B. (Dept. Biology, California State Univ., Northridge, Calif. 91324). *Exp. Cell Res.* 92(1):122-126; 1975.

Evidence is presented suggesting that teratoma cell adhesion factor (TAF) contains terminal D-galactosyl residues which are functionally involved in TAF cell binding activity. TAF was isolated from the peritoneal fluid of 127/J mice bearing mouse ascites teratoma. Activity of TAF was measured as aggregation of teratoma cells. Purified β-galactosidase from *E. coli* and bovine liver totally destroyed TAF aggregation promoting activity. Experiments with Azocoll indicated that the enzymes were not contaminated with protease. *O*-Nitrophenyl-β-D-galactopyranoside, a β-galactosidase substrate, prevented the enzyme from inactivating TAF suggesting that the enzyme catalysed removal of D-galactose residues from the TAF molecule. Cells treated with the enzyme, followed by washing, aggregated fully with TAF, suggesting that the enzyme catalysed removal of D-galactosyl residues from the TAF molecule and not from the cell surface. When the teratoma cells were preincubated with a variety of monosaccharides, followed by addition of TAF, D-galactose was the most active TAF-mediated aggregation inhibitor. Sugar acids were also effective inhibitors, though the effects of these

compounds may be due to non-specific charge properties. Protein may be the carrier of the galactose-containing carbohydrate involved in TAF activity.

1765 DIFFERENT ULTRAVIOLET DNA ENDONUCLEASE ACTIVITY IN HUMAN CELLS. (Eng.) Duker, N. J. (New York Univ. Medical Center, New York, N. Y. 10016); Teebor, G. W. *Nature* 255(5503):82-84; 1975.

Cells from patients with xeroderma pigmentosum (XP) seem to be excision-repair defective mutants and have been postulated to lack the UV endonuclease activity which catalyzes the incision of DNA adjacent to the pyrimidine dimer, the first step in the excision repair mechanism for UV-irradiated DNA. UV endonuclease activity in XP skin fibroblasts from four genetic complementation groups was assayed by measuring the conversion of UV-irradiated superhelical PM II phage DNA to the nicked form. Control cells were normal adult and fetal skin fibroblasts, WI-38 fetal lung cells, and HeLa cells. All cell preparations contained UV endonuclease activity as demonstrated by the nicking of irradiated superhelical DNA on both alkaline sucrose gradients and neutral cesium chloride gradients. HeLa cell activity increased from 12.5 to 75 μg protein/reaction mixture. WI-38 activity peaked at a similar protein content and was 70% that of HeLa. None of the XP or normal skin fibroblasts had activity greater than 40% that of HeLa, even at a protein content of 240 μg. These results demonstrate that an endonuclease activity is present to a similar degree in XP and normal skin fibroblasts. Thus, there is no correlation of activity with rates of UV repair synthesis, which suggests that the activity is not the UV repair endonuclease.

1766 PURIFICATION AND SUBUNIT STRUCTURE OF DEOXYRIBONUCLEIC ACID-DEPENDENT RIBONUCLEIC ACID POLYMERASE II FROM THE MOUSE PLASMACYTOMA, MOPC 315. (Eng.) Schwartz, L. B. (Div. Biological Biomedical Sciences, Washington Univ., St. Louis, Mo. 63110); Roeder, R. G. *Biol. Chem.* 250(9):3221-3228; 1975.

Class II RNA polymerase was purified to homogeneity from the mouse plasmacytoma, MOPC 315. Procedures were designed so that large quantities of enzyme could be quickly purified in very high yields. Three forms of RNA polymerase II were separated by electrophoresis under denaturing conditions, revealing distinct subunit structures for each enzyme form. Considerable purification was achieved by pelleting and discarding the chromatin, while the enzyme supernatant fraction was reserved. About 22% of the initial enzyme II activity was recovered after a total purification of about 25,000-fold. However, the loss of enzyme activity due to inactivation was only about 40% of the total initial activity. Subunit analysis of the enzymes was performed by electrophoresis. The more abundant enzymes II_a and II_b contained high molecular weight subunits. Low molecular weight subunit patterns were identical for II_e, II_c, and II_g. Possible reasons for this are variations in enzyme II structure, which could reflect difference in cell type. Another possibility is the present purification procedure, in which soluble RNA polymerase II was utilized; this may retain "α-like" enzyme components, which may not be bound to enzyme in transcription complexes. Greater structural complexity offers a greater possibility for the regulation of gene transcription. This may be particularly appropriate for enzyme II, which apparently selects and transcribes particular genes from a very large genetic repetoire.

1767 TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE ACTIVITY IN HUMAN LEUKEMIC CELLS AND IN NORMAL HUMAN THYMOCYTES. (Eng.) McCaffrey, R. (Cent. Cancer Res., Massachusetts Inst. Technol., Cambridge); Harrison, T. A.; Parkman, R.; Baltimore, D. *N. Engl. J. Med.* 292(15):775-780; 1975.

Peripheral WBC from normal and leukemic patients were assayed for activity of terminal deoxynucleotidyl transferase (TdT). Normal human thymus and stromal-free thymocytes were similarly assayed. WBC were harvested from venous blood by dextran sedimentation of RBC at 37 C or by the Latham Blood Processor leukapheresis system. Normal lymphocytes were recovered by the Ficoll-Hypaque gradient technique. Thymocyte suspensions were obtained from whole thymus and were fractioned on a discontinuous bovine serum albumin gradient. Enzyme extraction and purification were performed using phosphocellulose chromatography, and rechromatography was performed on some samples. TdT activity was detected in circulating blast cells from 11 of 13 patients with acute lymphoblastic leukemia and from one of four patients with chronic myelogenous leukemia in blast crisis. (Technical problems were noted in the assays performed on the cells of the two lymphoblastic leukemia patients showing negative TdT activity.) Activity was not detected in normal WBC or in patients with stable phase chronic myelogenous, acute myeloblastic, chronic lymphatic, or lymphosarcoma-cell leukemias. Both normal thymocytes and leukemic cells contain two forms of TdT, separable by phosphocellulose chromatography. The thymocyte and leukemic-cell enzyme have the same substrate and primer preference. The activity of TdT in a patient with chronic myelogenous leukemia in blast crisis suggests that the crisis may have been lymphoblastic rather than myeloblastic. TdT may be useful in classifying leukemic cells on a biochemical basis.

1768 ENHANCEMENT OF THE ELECTRICAL EXCITABILITY OF NEUROBLASTOMA CELLS BY VALINOMYCIN. (Eng.) Spector, I. (Weizmann Inst. Sci., Rehovot, Israel); Palfrey, C.; Littauer, U. Z. *Nature* 254 (5496):121-124; 1975.

Clone N1E-115 neuroblastoma cells (70-100 μm diameter) in the stationary phase of growth were exposed to valinomycin to determine any change in the electrical excitability of the cell lines. After the administration of 10^{-8} M valinomycin, a significant increase in the resting potential occurred along with a dramatic enhancement of electrical excitability. Valinomycin had no effect on intracellular K^+. An increase

o an increase in rate and amplitude after adminis-
ration of a depolarizing stimulus and threshold po-
ential was also observed after antibiotic administra-
ion. This observation suggests that valinomycin
ctiviates elements involved in fast inward movements
f intracellular Na^+ ions. The antibiotic did en-
ance electrical excitability of neuroblastoma cells.
urther investigation with other antibiotics is sug-
ested.

769 PSYCHOLOGICAL ATTRIBUTES OF WOMEN WHO DEVELOP BREAST CANCER: A CONTROLLED STUDY. Eng.) Greer, S. (King's Coll. Hosp., London, England); Morris, T. *J. Psychosom. Res.* 19(2):147-153; 975.

 psychological investigation of 160 women hospitalized for breast tumor biopsy was carried out by means of detailed structured interviews and standard tests. nterviews and testing were conducted on the day before operation, without knowledge of the provisional iagnosis. Information obtained from patients was erified in almost all cases by separate interviews ith husbands or close relatives. Statistical comparisons between 69 patients found to have breast ancer and a control group comprising the remaining 1 patients with benign breast disease showed a significant association between the diagnosis of breast ancer and a behavior pattern, persisting throughout dult life, of abnormal release of emotions. This bnormality was, in most cases, extreme suppression of anger and, in patients over 40, extreme suppression of other feelings. In patients under 40, 60% showed abnormal release of anger as opposed to 16% of the controls. In patients between 40 and 49, these values were 72% and 22%, respectively; in patients over 50, the values were 67% and 45%, respectively. Extreme suppression of emotions, though much less common, also occurred in a higher proportion of cancer patients than controls. The significant association between breast cancer and abnormal release of emotions were consistent when the results obtained by three interviewers were analyzed separately; this indicates that there was a reasonable degree of reliability in these findings.

1770 LOW MOLECULAR WEIGHT RNA SPECIES FROM CHROMATIN. (Eng.) Marzluff, W. F., Jr. (Dept. Chemistry, Florida State Univ., Tallahassee Fla.); White, E. L.; Benjamin, R.; Huang, R. C. C. *Biochemistry* 14(16):3715-3724; 1975.

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1776 METHYLATION OF DNA IN HUMAN NORMAL AND LEUKAEMIC WHITE CELLS. (Eng.) Malec, J. (Inst. Haematology, ul. Chocimska 5; 00-957 Warszawa, Poland); Wojnarowska, M.; Kornacka, L. *Acta Biochim. Pol.* 21(3):291-297; 1974.

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The Secretary of Health, Education and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1977.

NOTE

Journal names are abbreviated according to the list of abbreviations used by *Index Medicus*. For journals not covered by *Index Medicus*, the abbreviations found in *Chemical Abstracts Service Source Index*, 1907-1974 Cumulative, are used. New journals are verified in *New Serial Titles* and abbreviated according to *International Standard ISO 833*. An asterisk indicates the author to address (other than the primary) in requesting reprints.

LANGUAGE ABBREVIATIONS

Afr.	Afrikaans	Ind.	Indonesian
Ara.	Arabic	Ita.	Italian
Bul.	Bulgarian	Jpn.	Japanese
Chi.	Chinese	Kor.	Korean
Cro.	Croation	Lav.	Latvian
Cze.	Czech	Lit.	Lithuanian
Dan.	Danish	Nor.	Norwegian
Dut.	Dutch	Pol.	Polish
Eng.	English	Por.	Portuguese
Est.	Estonian	Rum.	Rumanian
Fin.	Finnish	Rus.	Russian
Fle.	Flemish	Ser.	Serbo-Croatian
Fre.	French	Slo.	Slovak
Geo.	Georgian	Spa.	Spanish
Ger.	German	Swe.	Swedish
Gre.	Greek	Tha.	Thai
Heb.	Hebrew	Tur.	Turkish
Hun.	Hungarian	Ukr.	Ukrainian
Ice.	Icelandic	Vie.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

A	angstrom(s)	M	molar
ACTH	adrenocorticotropic hormone	mM	millimolar
ADP	adenosine diphosphate	μM	micromolar
AMP	adenosine monophosphate	mOsm	milliosmolar
ATP	adenosine triphosphate	mEq	milliequivalents
BCG	Bacillus Calmette Guerin	min	minute(s)
bid	twice daily	mo	month(s)
C	degrees centigrade	MTD	maximum tolerated dose
cal	calorie(s)	N	normal concentration
kcal	kilocalorie(s)	NAD	nicotinamide adenine dinucleotide
cc	cubic centimeter(s)	NADH	reduced nicotinamide adenine dinucleotide
Ci	curie(s)	NADP	nicotinamide adenine dinucleotidephosphate
mCi	millicurie(s)	NADPH	reduced nicotinamide adenine dinucleotide-phosphate
μCi	microcurie(s)		
cm	centimeter(s)	ng	nanogram(s) (10^{-9})
CNS	central nervous system	od	once daily
cpm	counts per minute	Pa	ambient pressure
dl	deciliter(s)	PAS	periodic acid-Schiff
ml	milliliter(s)	pg	picogram(s) (10^{-12})
μl	microliter(s)	pgEq	picogram equivalent
DNA	deoxyribonucleic acid	po	orally
ED_{50}	median effective dose	ppb	parts per billion
EDTA	ethylenediamine tetraacetic acid	ppm	parts per million
ESR	erythrocyte sedimentation rate	qid	four times daily
g	gram(s)	qod	every other day
kg	kilogram(s)	QO_2	oxygen quotient
mg	milligram(s)	R	roentgen(s)
μg	microgram(s)	RBC	red blood cells (erythrocytes)
Hb	hemoglobin	RNA	ribonucleic acid
hr	hour(s)	sc	subcutaneous
ia	intra-arterial	sec	second(s)
ic	intracerebral	SGOT	serum glutamic-oxalacetic transaminase
icav	intracavitary	SGPT	serum glutamic-pyruvic transaminase
id	intradermal	SRBS	sheep red blood cells
ILS	increased life span	TCD	tissue culture dose
im	intramuscular	TCD_{50}	median tissue culture dose
ip	intraperitoneal	tid	three times daily
ipl	intrapleural	U	unit(s)
it	intratumorous	mU	milliunit(s)
IU	International Unit	UV	ultraviolet
iv	intravenous	vol	volume
K_m	Michaelis constant	WBC	white blood cells (leukocytes)
LD	lethal dose	wk	week(s)
LD_{50}	median lethal dose	wt	weight
m	meter(s)	x	times
mm	millimeter(s)	yr	year(s)

CONTENTS

801 CARCINOGENIC N-NITROSO COMPOUNDS. (Eng.) Lijinsky, W. (Biology Div., Oak Ridge latl. Lab., Oak Ridge, Tenn. 37830); Singer, G. M.; 'aylor, H. W. *Proc. Int. Cancer Congr. 11th.* 'ol. 3 *(Cancer Epidemiology, Environmental Factors).* 'lorence, Italy, October 20-26, 1974. Edited by ucalossi, P.; Veronesi, U.; Cascinelli, N. New 'ork, American Elsevier, 1975, pp. 44-47.

'he occurrence and *in vivo* formation of N-nitroso ompounds is discussed. While local and systemic umors are found to arise in every organ and tissue nd from most types of cells in response to treatent of animals with some nitroso compound, organ pecificity is shown. In addition, the affected rgan or tissue often varies with dose and length f treatment and the physiological state of the rgan. Most types of tumors found in man are easily nd regularly reproduced in experimental animals *ia* N-nitroso compounds; however, striking differnces in species specificity preclude any certain xtrapolation to human cancers. There are very 'ew reports of the natural occurrence of N-nitroso ompounds; two reported are streptozotocin and -methylnitrosaminobenzaldehyde. Limited industrial se of dimethylnitrosamine and other nitroso comounds is noted, but the greatest concern lies in he common occurrence and use of nitrites and nitates as fertilizers and food additives. The most ignificant source of nitroso compounds is their ormation in the stomach, a highly favorable milieu or nitrosation of secondary and tertiary amino ompounds; such nitroso compounds are likely to orm from the interaction of residues of agriculural chemicals, food additives, or drugs. However, ssessments are complicated by chemical factors overning the formation of N-nitroso compounds, ncluding the structure of the amine, the concentraion-dependent reaction rate, and the effects of ubstituent groups. While the existence of (cancer) pidemiological evidence of the effect of exposure o N-nitroso compounds is suggested, the first emonstration of the carcinogenic potential of -nitroso compounds formed from ingested nitrite nd amines was revealed in the induced esophageal umors of rats chronically fed methylbenzylamine nd nitrite. Tumors in liver, lung, nervous system, nd nasal cavity were also induced in experimental nimals by simultaneous feeding of amines and itrite. While a powerful tumorigenic effect is bserved with aminopyrine and nitrite, not all mines are capable of forming nitroso compounds o an extent sufficient for tumor induction. Howver, an accumulated response is also possible. 'herefore, the reduced ingestion of amines or niitrite and the administration of drugs at times ther than immediately before or after meals is ecommended. (17 references)

802 METABOLIC ACTIVATION OF POLYCYCLIC HYDROCARBONS. (Eng.) Sims, P. (Royal ancer Hosp., Fulham Road, London, England). *Proc. nt. Cancer Congr. 11th.* Vol. 2 *(Chemical and 'iral Oncogenesis).* Florence, Italy, October 20-26, 1974. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 9-14.

Polycyclic hydrocarbon reactions capable of yielding active metabolites are reviewed. Noting that polycyclic hydrocarbons are metabolized by NADPH-dependent mono-oxygenases, the major routes of benz[a]anthracene metabolism have been illustrated. Three epoxides are suggested as intermediates, subject to the actions of an epoxide hydrase, epoxide reductase, glutathione S-epoxide transferase, plus spontaneous rearrangement. While there is direct evidence for the formation of "K-region" epoxides from polycyclic hydrocarbons by rat liver and lung and by human lung, both "K-region" and "non-K-region" epoxides are synthesized; the latter are less stable. There is evidence that the "K-region" epoxides of many hydrocarbons are formed *via* cellular metabolism, are capable of reacting with cellular macromolecules, and are biologically active in numerous test systems. In addition, recent evidence has suggested that some of the dihydrodiols, e.g. derived from benz[a]anthracene, 7-methylbenz[a]anthracene, and 7,12-dimethylbenz[a]anthracene, are further metabolized by microsomal mono-oxygenases to products that react with glutathione, water, or nucleic acids. The formation and metabolic reactions of the diol-epoxides are described. Comparisons between the products obtained from the DNA of cells treated with aromatic hydrocarbons and those from DNA that was reacted with the related "K-region" epoxide have shown that, in each case, the products are different. Comparisons of the products from DNA of cells treated with benz[a]-anthracene and those from DNA that was reacted with the related 8,9-dihydrodiol-10,11-oxide have revealed their elution in similar column fractions and the lack of thin layer chromatography separation in two solvent systems. The results suggest that diol-epoxides may be involved in covalent binding to cellular DNA, but show that not all metabolically-formed dihydrodiols are converted into diol-epoxides that react with DNA. It is further suggested that such activation of polycyclic hydrocarbons may involve three stages: (1) metabolism on aromatic bonds, (2) hydration of the "non-K-region" epoxides thus formed, and (3) metabolism of the olefinic double bonds of those "non-K-region" dihydrodiols, forming diol-epoxides. (21 references)

1803 ANIMAL DATA ON PLUTONIUM TOXICITY. (Eng.) Thompson, R. C. (Battelle-Pacific Northwest Labs., Richland, Wash. 99352). *Health Phys.* 29(4):511-519; 1975.

Animal studies on the delayed effects of low-level plutonium exposure are reviewed. The inability to precisely simulate human conditions, and the uncertainties in extrapolating animal data to man are acknowledged. The cancer incidence appears to be the most sensitive measure of long-delayed effect of internally deposited plutonium. Bone cancer incidence data revealed a significantly increased incidence at the 30 rad level, the permissible occupational ex-

posure limit which would be accumulated over 50 yrs. Dogs were most frequently affected at all experimental doses, while increased osteosarcoma incidence was noted in all exposed groups. Studies of radium and plutonium distribution in bone indicate the 5-10 times greater toxicity of ^{229}Pu results from its more hazardous localization on bone surfaces. Despite the varying influence of the chemical and physical nature of the material inhaled, dogs also have the highest frequency of plutonium-induced lung cancer. Independent studies indicate a highest lung cancer incidence associated with lower dose ranges and uniform ^{238}Pu distribution. The liver appears to be less radiosensitive than bone and lung; liver cancers show a longer latent period following plutonium exposure. While no critical effects on lymph nodes have been noted, one of the most sensitive effects of inhaled plutonium is the reduction in numbers of blood lymphocytes. Acute plutonium lethality data in the rat show no marked differences in the adult, weanling, or newborn. However, ^{224}Ra has been found to be twice as effective in producing bone cancer in children as in adults. Some consideration is also given to the psychological effects of plutonium exposure. (25 references)

1804 HERPESVIRUS-DNA IN HUMAN TUMOR CELLS. (Eng.) zur Hausen, H. (Institut für Klinische Virologie der Universität Erlangen-Nürnberg, 852 Erlangen, Loschgestrasse 7, West Germany). *Proc. Int. Cancer Congr. 11th.* Vol. 2 *(Chemical and Viral Oncogenesis).* Florence, Italy, October 20-26, 1974. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 268-271.

Studies on the persistence of Epstein Barr (EB) viral DNA in human tumor tissue are summarized, and the presence of genomes of other herpesviruses in human tumors is discussed. With two possible exceptions, all 80 African Burkitt lymphomas tested *via* DNA-DNA or DNA-cRNA hybridizations contained EBV DNA. While usually at a high genome equivalent per cell ratio, the number of genome equivalents varied from three or four to 200. In contrast, all American Burkitt lymphomas tested for EBV DNA thus far have yielded negative results, but have revealed antibodies against EB viral antigens. Lymphoblastoid lines originating from Burkitt's lymphoma usually produce EB viral antigens and particles in a small percentage of cells, thus showing that at least part of the genomes persist nonfragmented and without deletions. The presence of EB viral DNA is also repeatedly confirmed in nasopharyngeal carcinomas; *in situ* nucleic acid hybridizations show its almost exclusive location in the nuclei of epithelial cells. Nasopharyngeal carcinomas obtained from patients from various parts of the world do not reveal differences in their EBV DNA content; the tumors thus appear more homogeneous than Burkitt lymphomas. However, EB viral DNA is not found in any other human malignancy, except for some unconfirmed biopsies of human melanomas. Studies on the state of EB viral DNA within human tumor cells suggest a plasmid state or an alkali-labile association of viral with host cell DNA. DNA-cRNA hybridization testing of 50 tumors revealed herpes simplex virus type two (HSV 2) DNA within only one tumor biopsy. Studies have revealed the persistence of herpes simplex virus type one predominantly within trigeminal ganglia, whereas HSV 2 is reported to reside within the sacral ganglia. Attempts to demonstrate DNA of other human pathogenic herpesviruses within human tumor material have been unsuccessful. While a relationship of herpes simplex viruses to human cancer is not yet established, the EB virus remains the prime candidate. (19 references)

1805 THE IMPORTANCE OF NON-UNIFORM DOSE-DISTRIBUTION IN AN ORGAN. (Eng.) Richmond, C. R. (Biomedical and Environmental Sciences, Holified Natl. Lab., Oak Ridge, Tenn.). *Health Phys.* 29(4): 525-537; 1975.

Numerous experiments on the effects of a non-uniform dose-distribution of plutonium and other actinide elements are cited, described, and/or summarized. A discussion of the attainment of a dosimetric basis for radiological protection notes two problems; the determination of the significant radiation dose, and of its early and late effects on the tissues and on the entire organism. The activity and number of various-sized particles required to deliver the occupational maximum for two nuclides of plutonium are calculated, and the relationship of particle size to the number of cells at risk is discussed. Studies on rat skin tumor response to various kinds and patterns of radiations are described, and the factors of dosage and penetrating power are considered. Other studies have noted the effects of ^{90}Sr beta particle radiation on rat skin, intratracheal ^{210}Po instillation in Syrian golden hamsters, $^{144}CeCl_3$ intratracheal injection into rats, and implantation of ^{90}Sr-containing glass beads into rat lungs. Further experiments on the "hot particle" problem have employed ^{35}S, $^{90}SR/^{90}Y$, ^{144}Ce, and ^{238}Pu. It is concluded that plutonium distributed nonuniformly in the lung is no more hazardous that the same amount uniformly distributed. Data suggest that the potential hazard increases as dispersion becomes more uniform. (41 references).

1806 VIRUSES AND BREAST CANCER. (Eng.) Moore, D. H. (Inst. Medical Res., Camden, N.J. 08103). *Proc. Int. Cancer Congr. 11th.* Vol. 2 *(Chemical and Viral Oncogenesis).* Florence, Italy, October 20-26, 1974. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 291-294.

The use of mouse mammary carcinoma(s) as a model in the study of mammary tumor etiology is discussed, and evidence for a human mammary tumor virus is presented. There are recognized differences in the expression of mammary carcinoma in man and mouse; it is also recognized that breast cancer is not a uniform disease within the same species. The mouse mammary tumor virus may be transmitted *via* the milk (strains developing early tumors) or

a the sex cells (strains developing fewer tumors te in life). Breast cancer in human can also divided into two general types, i.e., premeno- usal and postmenopausal. Although entirely rcumstantial, evidence indicates that a virus milar to that which causes breast cancer in mice ists in human milk and human breast tumors. The rphology of the virus is different from any other ructure in nature. The amount of virus produced the human breast is very small compared to the use, and is usually destroyed by factors in human lk. Nevertheless, an occasional virion indis- nguishable from the mouse mammary tumor virus found in human milk. The cross hybridizations nucleic acids from the two species is demonstra- d, and complementary DNA probes reveal that 30% human mammary tumors have RNA that hybridize to 80% of the DNA probe. Melting curves indi- te that the amount of mismatching is less than ; thus, identical base sequences in the nucleic id of human mammary tumors and of mouse mammary mor virus is indicated. Numerous cross species munological tests show that virus-rich mouse mors and cells react in immunofluorescence and munoelectron microscopy tests with sera from me women with breast cancer of fibrocystic masto- thy. Variants of the macrophage migration inhi- ition technique also provide results indicating e existence of one or more antigens common to e mammary tumor virus and human breast tumors. ree manners of viral expression and tumor develop- ent are found in mice. Strains with a 10-40% mor incidence late in life have been successfully munized with formalin-killed virus given im at 6 wk of age; a single immunization greatly de- eases the expression of viral antigen (60-80%), d delays the development of tumors, and greatly duces the incidence. A cellular immune mechan- m is postulated as the basis for this protection. e many indications of cross-antigenicity between use viruses and the postulated human breast can- r virus suggest the feasible use of mouse virus viral antigens for the prevention of human breast ncer. (11 references)

07 SPLEEN FOCUS-FORMING VIRUS IN FRIEND AND RAUSCHER LEUKEMIA VIRUS PREPARATIONS. ng.) Steeves, R. A. (Albert Einstein Coll. Med., onx, N.Y.). *J. Natl. Cancer. Inst.* 54(2):289-297; 75.

e biologic properties of spleen focus-forming virus FFV) in Friend and Rauscher leukemia virus prepar- ions are compared with those of murine sarcoma virus uSV). Both SFFV and MuSV are defective for virus plication but not for cell transformation; each quires a helper murine leukemia virus (MuLV) for cus formation. Recent experiments suggest a poss- le, though tentative, similarity between the RNA mponents of SFFV and MuSV. The viruses can be dis- nguished in part by their different pathogenicities. SV induces sarcomas in infected mice by transform- g fibroblasts and also by evoking a proliferative st response; the tumors often regress in immunolo- ically competent hosts. SFFV, which replicates in and transforms hematopoietic cells *in vivo*, does not transform mouse fibroblasts. This virus induces erythroleukemia and/or polycythemia. The induction of differentiating tumor colony-forming cells (CFC) and of autonomous CFC by SFFV is described. The former is the dominant cell in early Friend disease, while the latter gradually arises from the differentiating cells, perhaps by tumor progression. SFFV is a potent immunodepressive agent, and this may contribute to its rapid lethality in adult mice, in contrast to MuSV. Another difference between SFFV and MuSV is the genetic control of host resistance and susceptibility to these viruses. Though the disease induced by either virus can be influenced by an *H-2*-associated gene and the *Fv-1* gene, the *Fv-2* gene is specific for the expression of SFFV. Finally, SFFV and MuSV differ with respect to the specificity of agents that will provide a helper function. In addition to MuLVs, feline leukemia virus and similar viruses from hamsters and rats increase the focus-forming efficiency of MuSV in tissue cultures derived from the same species as the helper viruses. With SFFV, the host species cannot be changed, but helper activity is detected among an even broader range of samples. Rauscher SFFV differs from most Friend strains by not inducing polycythemia and by inducing extensive splenic necrosis and development of blood-filled sacs in the spleen of susceptible mouse strains. A proposed terminology is outlined for the Friend and Rauscher virus complexes and their components. (100 references)

1808 CELL BIOLOGY OF AGING. (Eng.) Hayflick, L. (Stanford Univ. Sch. Medicine, Stanford, Calif. 94305). *Bioscience* 25(10):629-637; 1975.

The predestination of normal somatic cells to undergo irreversible functional decrements interpreted as aging is discussed. Initial experiments culturing chick heart fibroblast cells *ad seriatum* infer that aging *per se* is not the result of events occurring at the cellular level. However, subsequent experiments have indicated that cultured normal human embryonic fibroblasts undergo a finite number of serial subcultivations (50±10), and that death is an inherent property of the cells. Frozen storage of human diploid cell strains arrests the cells at a particular population doubling level, but does not influence the total number of expected doublings. An inverse relationship is noted between donor age and the subsequent *in vitro* proliferative capacity of the cultured cells. Likewise, fewer doublings were obtained by cells from donors with Werner's syndrome and progeria. The latent period of explanted cells is also inversely correlated with donor age. A positive correlation is suggested between population doubling potential of cultured fibroblasts and mean maximum species lifespan. While tangential and logarithmic cell divisions are considered, the acquisition of the potential for unlimited cell division or escape from senescent changes is achieved only by cells which have acquired at least some properties of cancer cells. Despite the finite capacity for replication, it is suggested that other functional losses occurring in cells prior to the cessation of

division capacity produce the physiological decrements. Studies on the exchange of genetic information in protozoa suggest a senescence phenomenon attributed to the nonequivalency of daughter cells. Such an organ clock is assigned to the cell nucleus; the exchange of genetic information may then serve to reprogram or reset a more perfect biological clock guaranteeing species survival but death of the individual animal. (91 references)

1809 AN INTERPRETATIVE REVIEW: SURFACE FEATURE OF NORMAL AND LEUKEMIC LYMPHOCYTES AS SEEN BY SCANNING ELECTRON MICROSCOPY. (Eng.) Polliack, A. (Hadassah Med. Sch., Jerusalem, Israel); De Harven, E. *Clin. Immunol. Immunopathol.* 3(3):412-430; 1975.

The surface architecture of normal and leukemic lymphocytes prepared by critical point drying techniques was examined by scanning electron microscopy (SEM). Normal and leukemic cells had similar surface features; lymphocytes were spherical and contained varying numbers of microvilli, while monocytes had few microvilli and displayed ridges and ruffled membranes The "hairy cells" of leukemic reticuloendotheliosis resembled monocytes more than lymphocytes, although some cells shared features of both cell types. Thymus cells and thymus-derived lymphocytes had fewer microvilli and were generally smoother than bone marrow-derived B lymphocytes, which showed moderate to markedly villous surfaces. However, the surface of T cells became more villous during rosette formation in response to sheep RBC, making the SEM identification of overlapping B and T lymphocytes difficult without parallel immunologic identification. About 85 cases of acute and chronic lymphocytic leukemia (CLL and ALL) and related lymphoproliferative disorders were studied by SEM. Almost all CLL cases were B-derived and all but one of six ALL cases had no surface markers. Whereas ALL cells and cells of a single case of T-cell cancer were predominantly smooth, cells in the majority of CLL cases were moderately to markedly villous. About 20% of CLL lymphocytes showed an equal number of smooth and villous cells and in eight cases smooth cells predominated. These results indicate that SEM is a useful diagnostic tool for distinguishing among CLL, "hairy cell" and monocytic leukemias. Its value in ALL (or undifferentiated leukemias) and in T-cell leukemias can be determined only after study of a larger number of cases. (36 references)

1810 CROHN'S DISEASE: ASSOCIATED DISEASES. (Eng.) Schofield, P. F. (15 St. John St., Manchester M3 4DG, England). *Dis. Colon Rectum* 18(3): 192-193; 1975.

The underlying mechanisms in three conditions associated with Crohn's disease, i.e. renal stones, gallstones, and intestinal malignancy, are reviewed. Whereas gross, bilateral urinary tract obstruction is unusual with Crohn's disease, an association with minor right-sided obstructive renal disease is shown in a large proportion of the patients. It is suggested that in addition to the other changes in urinary composition occurring after colectomy, renal stones associated with Crohn's disease develop due to stasis. A statistically significant predominance of right-sided renal colic is observed both pre- and postoperatively. The high incidence of gallstones and pancreatitis associated with Crohn's disease is suggested to be due to disturbed bile salt absorption in the terminal ileum; the combined incidence of pre- and postoperative gallstones is reported as 10%. The relationship of malignant intestinal disease to Crohn's disease is reported at a 1% incidence. Of an approximate 1,000 cases of Crohn's disease, ten are reported to have had coincident small intestinal or colonic malignant tumors. In four of these cases, the association of Crohn's disease with malignancy is apparently coincidental: colonic carcinomas developed but not in apparently involved bowel. In addition to a higher incidence of ileal malignancy associated with long-standing ileal Crohn's disease, the risk seems increased by production of a surgical blind loop. Postulating that some carcinogenic alterations occur, two probabilities are suggested: (1) intraluminal stasis, due to changed small intestinal microflora and consequent changes in lipid breakdown within the lumen, may occur and produce carcinogens, or (2) a change in the immunologic status of the individual may also produce carcinogenic alteration. (5 references)

1811 HODGKIN'S DISEASE; ITS HETEROGENEOUS NATURE AND POSSIBLE INFECTIOUS AETIOLOGY. (Eng.) Vianna, N. J. (Cancer Control Bureau, New York State Dept. Health, Albany, N.Y.). In: *Lymphoreticular Malignancies: Epidemiologic and Related Aspects.* Baltimore, University Park Press, 1975, pp. 13-47.

Epidemiologic studies and histologic features of Hodgkin's disease are reviewed to determine the possible heterogeneous nature and infectious etiology of this disorder. Studies that compare adult and childhood forms are also presented. Cases of Hodgkin's disease were classified according to Rye's system, which categorizes the disorder into four subtypes. International comparisons of the incidence of Hodgkin's disease and analyses of factors such as sex, socio-economic status, and urban-rural differences suggest an epidemiologic heterogeneity for different age groups. Differences in sex ratios for various age groups also indicated heterogeneity; the disorder occurred with greater frequency in males, regardless of the age incidence pattern. Socio-economic factors appeared closely bound to variations in incidence and sex rates in Hodgkin's disease. It is suggested that four probes should be used to evaluate this disease in the child and the adult: (a) the possible effect of prior tonsillectomy; (b) seasonality, or birth month as possible indicators of some natal and/or prenatal influence; (c) human histocompatability system (HL-A, for determining antigen distribution in different age groups, regionally and internationally); and (d) two tumor-associated antigens, F (fast) and S (slow) antigens, which have been found in diseased lymph nodes and spleen. Evidence that suggests Hodgkin's disease is infectious in nature is presented. Epidemiologic features were found to be inconsistent

ith an infectious etiology. The strongest suspi-
ion of horizontal transmission was derived from
tudies of three different groupings of patients
ith Hodgkin's disease living in Albany, New York
n 1972. Rates among medical personnel and famil-
al aggregates were also studied for evidence of
orizontal transmission. Thus, the epidemiologic
nd histologic evidence suggests that environmental
actors play a major role in the etiology of Hodg-
in's disease. It is a dynamic disorder charac-
erized by different incidence patterns, interna-
ionally and regionally. The evidence also indi-
ates a heterogeneous nature of this disorder, es-
ecially between adult and childhood age groups.
everal hypotheses were advanced that suggest that
ome form of transmission may be important in adult
orms of Hodgkin's disease. (69 references)

312 EPIDEMIOLOGY OF VAGINAL ADENOCARCINOMA AND ADENOSIS: CURRENT STATUS. (Eng.)
eiss, K. (Sch. Public Health, Univ. Texas, Houston). *. Am. Med. Wom. Assoc.* 30(2):59-63; 1975.

vidence supporting the association of treatment ith diethylstilbestrol (DES) during pregnancy and aginal adenosis and adenocarcinoma in the offspring s presented. Although previously rare, between 970 and 1974 170 cases of clear-cell or "endome- rial" type adenocarcinoma of the vagina was repor- ed in women from 3-25 yr of age; 85% of these omen were exposed to DES or a similar synthetic strogen *in utero* at a critical stage of vaginal evelopment. DES had been given in doses ranging rom 2-222 mg daily to prevent miscarriage. It ppears that the critical time of exposure was the irst trimester of gestation, when as little as .5 mg DES/day for 12 days could cause the malig- ant transformation of a single, fetal cell in the uture vaginal tissue. That only female offspring re affected is explained by the Müllerian origin f the vagina. Offspring of women treated with ES after the first trimester, when the vagina of he fetus was already formed, are not affected. aginal adenosis is a rare condition, frequently ound to coexist with clear-cell adenocarcinoma. isual examination revealed vaginal adenosis in 8% of 34 DES daughters and no adenosis in controls. f 63 DES daughters examined with the colposcope, aginal adenosis was found in 91%, and extensive version of the columnar epithelium in the remain- ng 9%. In a larger study of 528 daughters, 65.5% ad vaginal lesions; 90% of these had a history f DES exposure and revealed gross and microscopic esions. The studies seem to support the hypothesis hat the mechanism of action of DES is in producing bnormalities of the vaginal epithelium. No envi- onmental factors were uncovered and no association etween maternal estrogen and cancer in sites other han the vagina were made. Whereas Schiller stain- ng is valuable in detecting clear-cell adenocar- inoma, the colposcope is cited for detection of he cancer in its preclinical stage. Treatment onsists of surgical extirpation, cauterization, nd radiation. Use of progesterone vaginal suppos- tories to reverse or avoid the onset of neoplasia s suggested. However, caution is urged because of the possibility of aggravating the course of genital cancers. An additional concern is the administration of DES as a postcoital contraceptive to already high risk DES daughters. (41 references)

1813 ETHNIC AND REGIONAL CONSIDERATIONS IN EPIDEMIOLOGY OF BREAST CANCER. (Eng.)
Macdonald, E. J. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.). *J. Am. Med. Wom. Assoc.* 30(3):105-113; 1975.

Regional and ethnic differences in breast cancer incidence are reviewed with emphasis on epidemiologic studies conducted in Texas. In Houston and San Antonio, the average five-yr age-adjusted incidence rate/100,000 (1962-1966) is highest in Anglo-American women, nearly half as high in Latin women and intermediate in blacks. For Houston, the incidence rates for these three ethnic groups are 74.3, 47.4 and 58.5, resp. Although all Texas studies by region show a low breast cancer incidence and mortality among Latin Americans, the rate exceeds that of the Japanese (one-fifth of the Anglo-American rate). In addition to great difference between ethnic groups in the same environment in the U. S., there are regional differences in breast incidence in the same ethnic group. While blacks in Houston have a 5-yr average incidence rate of 58.5/100,000, Nigerians with no Caucasian admixture have practically no breast cancer (<10/100,000 population). Similarly, Chinese and Japanese women in Hawaii have breast cancer rates substantially higher than rates in China or Japan. Comparative studies of estrogen profiles indicate that the estriol ratios of Orientals in Hawaii are more similar to those of Caucasians than to those of Oriental women in Asia. A Texas study for the period 1944-1958 revealed that the incidence of breast cancer in Abilene was significantly lower than that in either El Paso or Austin. Since the last two cities are characterized by shifting populations, it is hypothesized that high breast cancer rates in areas with mobile populations may be partly explained by a constant change of viruses in the environment which would preclude effective immunization against cancer-inducing viruses. With its three ethnic groups and with the population-based incidence of cancer and mortality known for 23 and 30 yr, resp., Houston may provide answers to the combined genetic, viral, demographic and ethnic implications of breast cancer. (18 references)

1814 CELL PROLIFERATION IN NORMAL UROTHELIUM AND UROTHELIAL TUMOURS. (Eng.) Cooper, E. H. (Dept. Experimental Pathology and Cancer Res., Univ. Leeds, Leeds, England). *Proc. Int. Cancer Congr. 11th.* Vol 1 *(Cell Biology and Tumor Immunology).* Florence, Italy, October 20-26, 1974. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 15-17.

Cell proliferation in normal urothelium, the response to injury, and the cell kinetics of bladder tumors are reviewed. A discussion of the unusual characteristics of the epithelium of the mammalian bladder

notes the cells adapted for urine contact, the polyploid tissue, the binucleate cells, and the remarkable tissue stability. While stathmokinetic studies in mice have confirmed the low proliferative activity, such stability is maintained only when the structural integrity of the tissue is intact. The response to injury results in focal or generalized hyperplasia, as typified by the massive response induced by a single systemic dose of cyclophosphamide. Chronic injury yields, progressively, random desquamation, considerable local variation, reversion to a diploid state, eventual reversion to the normal state, and polyploidy. In man, 90% of bladder tumors are transitional cell carcinomas (TCC); many can also be induced in experimental animals, but with a considerably varied response. General information on the growth of TCC in man is derived from [^{3}H]-thymidine labeling *in vitro* and stathmokinetic tests *in vivo*. A spectrum of TCC organization is noted, with the prognosis related to the grades and extent of invasion. A stathmokinetic analysis of proliferation of TCC in man is tabulated, and indicates a rising mitotic index corresponding to decreased differentiation. Analysis of 187 untreated or ineffectively treated TCC patients has revealed an average survival of 16.5 mo and a massive cell loss. (11 references)

1815 CELL KINETICS AND CANCER. (Eng.) Lala, P. K. (Dept. Anatomy, McGill Univ., Montreal, Canada). *Proc. Int. Cancer Congr. 11th.* Vol. 1 *(Cell Biology and Tumor Immunology).* Florence, Italy, October 20-26, 1975. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 29-35.

The dynamic histology of the tumor and the tumor-bearing host is analyzed. Dynamic cellular components of the tumor and the host are tabulated, and the significance of the vascular bed, the connective tissue stroma, and of hematogenous cells such as lymphocytes, monocytes, macrophages, and granulocytes is discussed. It is noted that tumor cells seldom constitute a homogeneous population, but rather may retain partial differentiation characteristics of the tissue of origin. Clonogenic cells, heterogeneous nonproliferative cells and persistent but quiescent tumor cells are also found. Effective tumor growth results from the discrepancy between cell production and loss; production rate depends on the duration of the mitotic cycle and the proportion of proliferative cells, while tumor cell loss may represent death, migration, and surface exfoliation. Hence studies of the dynamic histology of host cell renewal systems require knowledge of the renewing tissues of the host bearing the tumor, rather than a tumor-free host. A review of techniques employed for measuring various growth parameters discusses the estimation of growth rate, the measurement of mitotic cycle time, growth fraction, and stem or clonogenic fractions, and the indirect measurement of rate of cell loss. Studies of growth parameters in numerous experimental and a few human tumors indicate: (a) kinetically, tumors are comparable with growing cell renewal systems of the body, (b) the duration and variance of cell cycle time in tumors increases in the order of transplantable rodent tumors, induced or spontaneous tumors in laboratory animals, spontaneous tumors in man, and (c) micro-environment plays an important role in tumor growth. Cell cycle characteristics of some solid human cancers are tabulated and various relationships of cell cycle time and tumor doubling time are graphically represented. It is concluded that no one single parameter appears less variable than the others. (47 references)

1816 DIETARY FAT AND COLON CANCER. (Eng.) Wynder, E. L. (Am. Health Found., New York, N.Y.); Reddy, B. S. *J. Natl. Cancer Inst.* 54(1):7-10; 1975.

An association between cancer of the colon and dietary animal fat and/or cholesterol is suggested by the geographic and socioeconomic distribution of colon cancer, data on migrant populations, and retrospective studies of U. S. and Japanese patients with large-bowel cancer. The rate of colon cancer, particularly in the sigmoid region, in Japanese immigrants increases significantly as they adjust to American diets. This increase may be related to intake of beef, which contributes 35-45% of the total fat content of the American diet. Intrarectal instillation of two bile acids (deoxycholic and lithocholic acid) in rats significantly promoted the development of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced adenomas. Parallel studies *in vitro* indicated that these bile acids were more extensively degraded to microbial products by anaerobes isolated from Americans on a mixed Western diet than from American vegetarians. These data support the concept that different diets lead to typical microflora populations which control the metabolism of intraluminal compounds. One or a combination of active anaerobic strains, acting simultaneously or sequentially on cholesterol or bile acid metabolites or degradation products, could possibly form a co-carcinogen and/or a carcinogen. (34 references)

1817 GLYCEROLIPIDS AND CANCER. (Eng.) Snyder, F. (Medical Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, Tenn. 37830); Snyder, C. *Prog. Biochem. Pharmacol.* 10:1-41; 1975.

Data on specific glycerolipid classes in cancer are discussed and compared to those for normal tissues. Individual molecular species of glycerolipids, and tumor phospholipids containing choline and ethanolamine are especially noted. Thin layer chromatography is extensively used in the resolution of acyl, alkyl, and alk-1-enyl types of nonphosphorus glycerolipids. A discussion of the occurrence of ester and ether glycerolipids in neoplastic cells notes the elevated triacylglycerol levels found in various tumors, and the distinguishing alkyl ether analogues of triacylglycerols found in most neoplastic cells. Lipid extracts from tumors also reveal unique alk-1-

nyl analogues of triacylglycerols. However, no conclusions are reached on the presence of the ether nalogues of diacylglycerols and monoacylglycerols. espite the difficulties in the analysis of phospholipids, phospholipid values have been assessed for rain tumors, Novikoff hepatoma, Ehrlich ascites carinoma, mammary tumors, ovarian tumors, human cervial tumors, and various murine solid tumors. Subractions of ethanolamine and chlorine phospholipid lasses are analyzed, but no general relationships f the proportions of the subfractions have yet been etermined. Pathways for ester and ether glycerolipids, and their significance in neoplastic cells re evaluated. The formation of acyl (ester) bonds, lkyl (ether) linkages, and alk-1-enyl (plasmologen) inkages at the sn-1-position of glycerolipids are echanistically discussed. The sn-2-position is ound to participate in various acyl-transferase reactions, while reactions at the sn-3-position most ommonly involve phosphotransferases. Catabolic enymes attacking at the sn-1-, sn-2-, and sn-3-positions are also discussed. Regulation of glycerolipds and their precursors in cancer cells involves he biosynthesis of alkyldihydroxy acetone phosphate, he biosynthesis of fatty alcohols, and the enzymaic cleavage of ether lipids. It is concluded that ther lipid accumulation in tumor tissues results rom elevated enzymatic activites for ether lipid ynthesis, and decreased enzymatic degradative ctivities. (161 references)

818 RECENT BIOCHEMICAL FINDINGS IN LEUKAEMIA. (Ger.) Deutsch, E. (I. Medizinische Uniersitäts-Klinik, Siebensterngasse 2/I, Wien 62/VII, ustria); Höcker, P.; Pittermann, E.; Stacher, A.; ainer, H.; Moser, K. *Haematologia (Budap.)* 8(1-4): 5-42; 1974.

ecent achievements in the study of leukemia cell NA polymerases are reviewed. A DNA polymerase ound in leukemia cells was discovered to have the emplate characteristics of reverse transcriptase, nd to be able to read information from a natural eteropolymer RNA and to convert this information nto a DNA sequence. It was possible to separate his RNA-dependent DNA polymerase from the DNA olymerases of leukemia cells. The reverse trancriptase of myeloid leukemia has a considerably reater molecular wt than that of lymphatic leuemia. No such transcriptase was found in normal r phytohemagglutinin-stimulated leukocytes. An NA-dependent DNA polymerase with the typical temlate characteristics of reverse transcriptase was ound in a patient with pancytopenia and glutathione eductase deficiency at a time when thorough clinial and histochemical investigations of the peripheral blood and of the bone marrow detected no eukemia. The patient developed peroxidase-positive cute leukemia a little later. *In vitro* activation f the DNA-dependent DNA polymerases, and inhibition f the reverse transcriptase by rifamycin was oberved. (21 references)

819 PRENATAL EXPOSURE TO STILBOESTROL. (Eng.) Anonymous. *Med. J. Aust.* 1(12):373-374; 975. (5 references)

1820 DILEMMA OVER TREATING DES DAUGHTERS: GYNECOLOGISTS SPLIT ON WHAT TO DO ABOUT STILBESTROL-RELATED ADENOSIS. (Eng.) Anonymous. *Med. World News* 16(26):37; 1975. (No references)

1821 NOTES ON HORMONE-DEPENDENT TUMORS OF THE FEMALE GENITALIA. (Ita.) Rendina, G. M. (Ospedale S. Camille De Lellis, Pio lst. di S. Spirito e OO.P.R. - Roma, Italy). *Minerva Ginecol.* 27(2):148-157; 1975. (70 references)

1822 SMOKING AND LUNG CANCER. (Eng.) Jones, S. J. (No affiliation given). *Nurs. Mirror* 141(12):48-49; 1975. (3 references)

1823 ON SOME ASPECTS OF THE TOBACCO-CANCER PROBLEM. (Fre.) Hubert-Habart, M. (Fondation Curie-Institut du Radium, 26, rue d'Ulm, F 75231 Paris, Cedex 05, France). *Bull. Cancer (Paris)* 62(1):73-90; 1975. (59 references)

1824 ASBESTOS AS AN ENVIRONMENTAL CARCINOGEN. (Ger.) Jacob, G. (Bezirkskrankenhaus Karl-Marx-Stadt, DDR-90 Karl-Marx-Stadt, Zeisigwaldstr. 101, East Germany). *Z. Erkr. Atmungsorgane* 142(1):3-17; 1975. (85 references)

1825 OSHA PROPOSAL WOULD LOWER ASBESTOS EXPOSURE LEVEL, MAKE OTHER CHANGES. (Eng.) Anonymous. *Am. Paint Coatings J.* 60(21):7-8; 1975. (No references)

1826 ASBESTOSIS. (Ger.) Hany, A. (No affiliation given). *Ergeb. Inn. Med. Kinderheilkd.* 36:19-95; 1974. (263 references)

1827 THE VINYL CHLORIDE STORY. (Eng.) Moore, J. W. (East. Michigan Univ., Ypsilanti, Mich. 48197). *Chemistry* 48(6):12-16; 1975. (8 references)

1828 SOME ASPECTS OF THE DETECTION OF POTENTIAL MUTAGENIC AGENTS IN DROSOPHILA. (Eng.) Vogel, E. (Dept. Radiat. Genet. Chem. Mutagenesis, Univ. Leiden, Netherlands). *Mutat. Res.* 29(2):241-250; 1975. (48 references)

1829 CHARLOTTE AUERBACH AND CHEMICAL MUTAGENESIS. (Eng.) Sobels, F. H. (Dept. Radiat. Genet. Chem. Mutagenesis, Univ. Leiden, Netherlands). *Mutat. Res.* 29(2):171-180; 1975. (63 references)

1830 LEGISLATIVE AND TECHNICAL ASPECTS OF MUTAGENICITY TESTING. (Eng.) Mayer, V. W. (Div. Toxicol., Food Drug Adm., Washington, D.C.); Flamm, W. G. *Mutat. Res.* 29(2):295-300; 1975. (22 references)

1831 CIRCULATION OF CARCINOGENS IN THE ENVIRONMENT. (Rus.) Shabad, L. M. (No affiliation given). Moscow, Izd-vo "Meditsina", 1973, 367 pp.

1832 DIMENSION OF THE PROBLEM OF OCCUPATIONAL CARCINOGENESIS AND THE ROLE OF PREDICTIVE CARCINOGENICITY BIOASSAYS. (Eng.) Maltoni, C. (Istituto di Oncologia 'Felice Addarii' and Centro Tumori, Bologna, Italy). *Proc. Int. Cancer Congr. 11th.* Vol. 3 *(Cancer Epidemiology, Environmental Factors).* Florence, Italy, October 20-26, 1974. Edited by Bucalossi, P.; Veronesi, U.; Cascinelli, N. New York, American Elsevier, 1975, pp. 79-80. (4 references)

1833 METABOLIC RESEARCH. (Eng.) Mills, I. H. (No affiliation given). *Chem. Ind. (London)* (10):418-419; 1975. (No references)

1834 CAN WE LIVE WITH PLUTONIUM? THE ELEMENT OF THE LORD OF HELL? (Eng.) Tamplin, A. (Natural Resources Defense Council, Washington, D.C.); Cochran, T. *New Sci.* 66(951):497-501; 1975. (No references)

1835 NEW DATA FOR THE CHARACTERIZATION OF LYMPHOCYTES IN CHRONIC LYMPHATIC LEUKEMIA. (Ger.) Theml, H. (I. Medizinische Abeteilung des Stadtischen Krankenhauses Schwabing, 8 Munchen 40, Kolner Platz 1, West Germany); Huber, H. *Med. Klin.* 70(14):599-605; 1975. (63 references)

1836 HODGKIN'S DISEASE. (Eng.) Sinkovics, J. G. (Univ. of Texas System Cancer Center, Houston, Tex. 77025); Shullenberger, C. C. *Lancet* 2(7933):506-507; 1975. (32 references)

1837 THE IMMUNE SYSTEM AND MALIGNANCY: ANOTHER PERSPECTIVE. (Eng.) Bentley, H. P., Jr. (Kiwanis Cancer Center, Dept. Pediatrics, Univ. South Alabama, Mobile, Ala. 36617); Hughes, E. R.; Peterson, R. D. A. *J. Pediatr.* 87(3):503-504; 1975. (18 references)

1838 THE IMMUNOLOGICAL ASPECTS OF MALIGNANT DISEASE. (Eng.) Oliver, R. T. D. (St. Bartholomew's Hosp., London, England). *Practitioner* 214(1282):511-521; 1975. (64 references)

1839 HISTOPATHOLOGY OF CENTRAL NERVOUS SYSTEM LEUKEMIA. (Eng.) Price, R. A. (St. Jude Children's Res. Hosp., 332 N. Lauderdale, Memphis, Tenn. 38101). *Mod. Probl. Paediatr.* 16:80-98; 1975. (37 references)

1840 OF MOLES AND MALIGNANCY. (Eng.) Anonymous. *Br. Med. J.* 2(5963):106-107; 1975. (No references)

1841 SYMPOSIUM: CHARACTERIZATION OF ONCORNAVIRUSES AND RELATED VIRUSES--A REPORT. (Eng.) Dalton, A. J. (Natl. Cancer Inst., Bethesda, Md. 20014); Heine, U. I.; Melnick, J. L. *J. Natl. Cancer Inst.* 55(4):941-943; 1975. (No references)

1842 VIRAL LEUKEMIAS (PART II). (Spa.) Villaescusa, V. G. (Servicio de Hematologia, Ciudad Sanitaria Juan Canalejo, La Coruna, Spain). *Galicia Clin.* 47(3):200-221; 1975. (No references)

1843 NEOPLASTIC DEVELOPMENT. VOL. 2. (Eng.) Foulds, L. (No affiliation given). New York, Academic Press, 1975, 732 pp.

1844 CANCER PREVENTION. ITS BASIS, THEORIES AND PRACTICE. (Por.) Conde, J. (No affiliation given). *Arq. Patol.* 46(2/3):209-249; 1974. (No references)

1845 CHILDHOOD LYMPHOMAS. (Eng.) Vianna, N. J. (Cancer Control Bureau, New York State Dept. Health, Albany, N.Y.) In: *Lymphoreticular Malignancies: Epidemiologic and Related Aspects.* Baltimore, University Park Press, 1975, pp. 97-109. (47 references)

1846 THE PROBLEM OF THE OCCUPATIONAL ENVIRONMENT. (Eng.) Pittom, L. A. (Director of the Hazardous Substances Group of the Health and Safety Executive, Baynards House, 1 Chepstow Place, Westbourne Grove, London W2, England). *Chem. Ind. (London)* (18):768-770; 1975. (No references)

1847 THE CANCER PROBLEM. (Eng.) Cairns, J. (No affiliation given). *Sci. Am.* 233(5):64-72, 77-78; 1975. (No references)

1848 PROGRAMME OF THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER: EPIDEMIOLOGICAL APPROACH. (Fre.) Higginson, J. (Centre International de Recherche sur le Cancer, 150, cours Albert-Thomas, F 69008 Lyon, France). *Bull. Cancer (Paris)* 62(2):137-150; 1975. (No references)

1849 PREVALENCE AND SIGNIFICANCE OF DIGESTIVE DISEASE. (Eng.) Almy, T. P. (Dartmouth Med. Sch., Hanover, N.H.); Mendeloff, A. I.; Rice, D.; Lilienfeld, A.; Klarman, H.; Rawson, R.; Cunnick, W. R. *Gastroenterology* 68(5/Part 2):1351-1371; 1975. (3 references)

1850 DIETARY FIBER AND FOOD TECHNOLOGY [abstract]. (Eng.) Scala, J. (Thomas J. Lipton, Inc., 800 Sylvan Ave., Englewood Cliffs, N.J. 07632). *Cereal Foods World* 20(9):447; 1975. (No references)

351 DIET, INTESTINAL FLORA, AND COLON CANCER. (Eng.) Anonymous. *Nutr. Rev.* 33(5): 36-137; 1975. (No references)

352 DIETARY FIBER: BACK TO BASICS. THE ISSUES AND SOME CONSIDERATIONS WHY ODAY HIGH FIBER DIETS ARE POPULAR. (Eng.) nonymous. *Drug Ther.* 5(8):128-131; 1975. (No eferences)

353 DIETARY FIBER [abstract]. (Eng.) Kritchevsky, D. (Wistar Inst., Philadelphia, a. 19104). *Cereal Foods World* 20(9):447; 1975. No references)

1854 BIOCHEMISTRY OF BRAIN TUMORS. (Rus.) Wollemann, M. (No affiliation given). Budapest, Akademiai Kiado, 1974, 194 pp.

1855 CULTURED NEUROBLASTOMA AND NEURAL TUMORS: TOOLS FOR THE DEVELOPMENTAL BIOLOGIST. (Eng.) Herschman, H. (UCLA Lab. of Nuclear Medicine, Calif.) *UCLA Cancer Cent. Bull.* 2(6):5; 1975. (No references)

1856 ASSESSING HAZARDS FROM PROLONGED AND REPEATED EXPOSURE TO LOW DOSES OF TOXIC SUBSTANCES. (Eng.) Barnes, J. M. (Medical Res. Council, Toxicology Unit, Carshalton, Surrey, England). *Br. Med. Bull.* 31(3):196-200; 1975.

The possible long-term effects of exposure to low doses of toxic substances are discussed. In presenting an analogy with vitamins, it is suggested that while some trace substances carry out essential roles in whole-body metabolism, others may have detrimental effects. The mode of action and mode of disposal by the whole organism would then determine the danger and possible secondary effects of repeated low doses of such poisons. The need of such toxic substances to reach critical concentrations for competitive action is acknowledged. However, the specificity and reversibility of its adverse reactions in the tissues, and not the degree of water solubility, determines the safety level of repeated doses. The reaction rates also determine the nature of the pharmacological response. In evaluating the nature of the toxic injury, it is noted that the toxic effects of most organophosphorus inhibitors of cholinesterase are either immediately fatal or rapidly and totally reversible. However, delayed structural damage involving the central and peripheral nervous systems is also caused by tri-*o*-cresyl phosphate, suggesting a possible cumulative effect of exposure to low doses. Consideration of persistent organochlorine compounds notes the establishment of steady state body levels of DDT and dieldrin, the rapid metabolization of methoxychlor and endrin, and the accumulation of hexachloroxycyclohexane. Limited information on the mode of action of DDT and dieldrin indicates the ingestion of dietary residues has no adverse effects, as critical blood levels are not reached. In contrast to most organochlorine insecticides, data indicate that chlorinated dibenzodioxins and their toxic effects on liver cell membranes are persistent and cumulative. A dose-response of tumor formation by chemical carcinogens is suggested, and prolonged exposure is thus cautioned. Also, sensitized persons may exhibit severe and progressive responses to toxic compounds. (30 references)

1857 AN EXPERIMENTAL STUDY OF INTRAORAL CARCINOGENESIS IN RATS. (Eng.) Yamamura, T. (Tokyo Dent. Coll., Misakicho, Chiyoda-Ku, Tokyo 101, Japan); Nishida, Y.; Eda, S.; Shimono, M.; Yamane, H.; Tachikawa, T.; Koike, H.; Ichikawa, T.; Yoshida, M.; Watanabe, O.; Matsuyama, H. *Oral Surg.* 39(1):87-102; 1975.

A new method was presented for preparing artificial cecal pouches lined with mucous epithelium in the lower lips of five-week-old Wistar and Sprague-Dawley rats (120 g each). General anesthesia was induced by an i.p. injection of 50 mg/kg of 2.5% thiopental sodium and the mucous membrane of the lower lip was sliced to a 3 mm width in six places. Three splints (plastic boards, 0.5 x 10 x 3 mm) were applied to join wound surfaces and sutures were placed. During the third week after operation, 9,10-dimethyl-1,2-benzanthracene (DMBA), 20-methylcholanthrene (MC) and *N*-methyl-*N*'-nitro-nitrosoguanidine (NG) were administered. The dosages were: 0.5% mineral oil solution of DMBA to 50 Sprague-Dawley rats three times/week for periods ranging from 116-291 days, a crystal of MC to 25 Wistar and 28 Sprague-Dawley rats once/week for periods ranging from 72-616 days, and a crystal of NG to 26 Sprague-Dawley rats once/week for periods ranging from 117-223 days. These carcinogens produced: squamous-cell carcinoma, carcinoma *in situ*, papilloma, adenoma sebaceum, neurofibroma, fibroma, hemangioma and lymphangioma in the oral mucosa. Tissue changes in the mucous epithelium of the cecal pouches during the carcinogenic process were also reported.

1858 NEOPLASTIC TRANSFORMATION OF GUINEA PIG FETAL CELLS IN CULTURE INDUCED BY CHEMICAL CARCINOGENS. (Eng.) Evans, C. H. (Natl. Cancer Inst., Bethesda, Md.); DiPaolo, J. A. *Cancer Res.* 35(4):1035-1044; 1975.

Relationships between various cell properties and tumorigenic potential were studied in 24 cell strains derived from freshly isolated diploid strain 2 Sewall-Wright guinea pig fetal cells exposed *in utero* or directly in culture to a carcinogenic or a noncarcinogenic chemical during 4-24 months of continuous culture. Carcinogenic agents included benzo(*a*)pyrene, 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, diethylnitrosamine, aflatoxin B_1, and N-acetoxyacetylaminofluorene. Noncarcinogenic chemicals were acetone, pyrene, anthracene, and phenanthrene. Morphological alterations (including various degrees of loss of cell orientation) were seen in the cultures exposed for five days to carcinogens but not in controls; however, these morphologic changes did not appear until four or more months, and did not appear simultaneously with the capacity of transformed cells to grow as tumors in irradiated syngeneic newborn animals. Changes in plating efficiency, saturation density, doubling time, or chromosome alterations did not correlate with progressive tumor growth. Formation of colonies in 0.35% agar, however, did develop concurrently with the potential for neoplastic growth, and of all the cell properties examined it was the best indicator of the neoplastic state. Four to 18 months of culturing was required after exposure to the carcinogen.

1859 DNA REPAIR SYNTHESIS OF CULTURED HUMAN CELLS AS A RAPID BIOASSAY FOR CHEMICAL CARCINOGENS. (Eng.) San, R. H. C. (Cancer Res. Center, Univ. British Columbia, Vancouver, Canada); Stich, H. F. *Int. J. Cancer* 16(2):284-291; 1975.

The feasibility of detection of carcinogenic chemicals using DNA repair synthesis of cultured human fibroblasts as measured by an unscheduled tritiated thymidine incorporation was investigated. Nearly nondividing populations of fibroblasts obtained from skin-punch biopsies of 12 18- to 24-yr old men and women were kept for three days in an arginine-deficient medium. Eight different dilutions of the

test compounds were dissolved in dimethylsulfoxide or ethanol (not exceeding 1% and 5%, respectively) and added to the cultures for 1.5-5 hr. Tritiated thymidine (10 μCi/ml) was added for 1.5 hr immediately following exposure to the chemicals. Of 64 chemicals tested, 29 were proximate or ultimate carcinogens, 15 were precarcinogens that required metabolic activation, 16 were nononcogenic compounds and four were of unknown carcinogenicity. All directly acting carcinogens triggered a DNA repair synthesis, whereas no unscheduled tritiated thymidine incorporation was observed following the application of the 16 nononcogenic compounds. As a rule, the precarcinogens (without metabolic activation) do not elicit DNA repair synthesis. However, longer exposures and higher concentrations of the precarcinogens aflatoxin B_1 and sterigmatocystin gave unscheduled thymidine uptake. The results suggest the suitability of using repair synthesis as endpoint, and cultured human cells as subjects in a prescreening program for chemical carcinogens.

1860 THE METABOLISM AND DISPOSITION OF ANILINE IN THE ISOLATED BLOOD-PERFUSED LIVER OF THE RAT. (Eng.) Boobis, A. R. (Dep. Pharmacol., Glasgow Univ., Scotland); Powis, G. *Drug Metab. Dispos.* 3(2):63-68; 1975.

The metabolism and disposition of aniline in isolated, blood-perfused liver of 200 male albino Wistar rats (250 g) was studied. The perfusion medium (150 ml) consisted of washed, aged human erythrocytes in Krebs bicarbonate-buffered saline, pH 7.4, containing 3% bovine serum albumin and 0.15% glucose, gassed with air containing 5% CO_2. Aniline in the medium followed a biphasic decline and was removed rapidly over the first 60 min, but more slowly over the next 120 min. The main metabolite in the perfusion medium, an acid-labile conjugate of aniline, accounted, after 3 hr, for 33% of the aniline added. *p*-Aminophenol conjugates accounted for 13%. No free *p*-aminophenol was found in the medium and only small amounts were present in the bile. Aniline conjugates formed the major metabolites in the bile. Kinetic analysis of the data suggested a two-compartment model in which aniline is removed for excretion in the bile or for metabolism from the first compartment. The addition of SKF 525-A (0.2 mM), a drug metabolism inhibitor, completely blocked the formation of *p*-aminophenol, inhibited the formation of acid-labile conjugate by 62%, and increased the size of the second compartment of the kinetic model.

1861 EFFECT OF REPEATED EXPOSURE TO ANILINE, NITROBENZENE, AND BENZENE ON LIVER MICROSOMAL METABOLISM IN THE RAT. (Eng.) Wisniewska-Knypl J. M. (Inst. Occup. Med., Lodz, Poland); Jablonska, J. K.; Piotrowski, J. K. *Br. J. Ind. Med.* 32(1):42-48; 1975.

The effects of repeated exposure to aniline, nitrobenzene, and benzene on the rate of their microsomal metabolism were studied in male Wistar rats. The three compounds were administered s.c. at a rate of 5 and 50 mg/kg daily for a month, or were administered at a level of 150 mg/kg daily for three days. *In vitro* assays of the liver microsomal drug-metabolizing enzymes were then conducted. Hexobarbital sleeping time and the antipyretic activity of phenacetin were also used to evaluate the effects of the test substances on the rate of biotransformation in the intact animal. Exposure of the rats to aniline at 150 mg/kg for three days or at 50 mg/kg for a month stimulated the microsomal metabolism of this drug as evidenced by: an acceleration of the p-hydroxylation of aniline and N-demethylation of aminopyrine in the postmitochondrial liver supernatant, shortening of the sleeping time after hexobarbital, and reduction of the antipyretic effect of phenacetin. In the rats exposed to nitrobenzene at 50 mg/kg daily for a month, the nitroreduction of nitrobenzene and p-hydroxylation of aniline remained unaffected, as did the hexobarbital sleeping time; the antipyretic effect of phenacetin was, however, decreased. At 150 mg/kg for three days, nitrobenzene enhanced the activity of nitrobenzene nitroreductase. Exposure of the rats to benzene at 50 mg/kg daily for a month had no effect on the rate of hydroxylation of benzene, the N-demethylation of aminopyrine, or the antipyretic effect of phenacetin, although the hexobarbital sleeping time was prolonged. The microsomal metabolism of aniline, nitrobenzene, and benzene was stimulated and inhibited when the rats were pretreated with phenobarbital and SKF 525-A, resp. The results of this study may have some value for industrial toxicology.

1862 EFFECT OF TREATMENT OF RATS WITH SOME CHEMICAL CARCINOGENS ON THE STIMULATORY EFFECT OF ADRENALINE ON CYCLIC AMP ACCUMULATION IN LIVER SLICES. (Eng.) Christoffersen, T. (Inst. Pharmacology, Univ. Oslo, P. O. Box 1057, Blindern, Oslo 3, Norway). *Acta Pharmacol. Toxicol. (Kbh.)* 37(3):233-236; 1975.

Experiments were performed to test the effect of adrenaline on cyclic AMP formation in liver slices from male Wistar rats fed either 2-acetylaminofluorene (AAF, 0.025%) for 4-8 wk or a 0.05% solution of one of the following carcinogenic azobenzenes: 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB), 4-dimethylaminoazobenzene (DAB), or 2'-methyl-4-dimethylaminoazobenzene (2'MeDAB). Cyclic AMP levels in slices from control livers was slightly increased (about 35%) by the presence of adrenaline (5×10^{-5} M) during incubation. In contrast, adrenaline caused a 13-fold elevation of the cyclic AMP content in liver slices from rats fed AAF and a 4- to 5-fold increase of the nucleotide in slices from rats fed 3'MeDAB. A small increase in the response to adrenaline was also seen in slices from rats fed the moderately carcinogenic compound DAB. The effect of adrenaline on slices from rats fed 2'MeDAB did not differ from the effect on control slices. These findings may indicate that different carcinogens have similar actions on hepatic adenylate cyclase, and that their effectiveness in this respect parallels their order of potency as carcinogens. However, it is also possible that the more carcino-

genic substances are more reactive chemically, or yield reactive metabolite(s) more rapidly or in larger quantities, than less carcinogenic analogues.

1863 A COMPARISON OF THE EFFECTS OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE, 2-METHYL-4-DIMETHYLAMINOAZOBENZENE, AND 2-ACETYLAMINOFLUORENE ON RAT LIVER DNA STABILITY AND NEW SYNTHESIS. (Eng.)
Yager, J. D., Jr. (McArdle Lab. Cancer Res., Univ. Wisconsin, Madison); Potter, V. R. *Cancer Res.* 35 (5):1225-1234; 1975.

Early biochemical changes occurring in livers of rats that were fed various chemical carcinogens were investigated. Male Sprague-Dawley rats were subjected to partial hepatectomy and subsequently given multiple im injections of radioactive thymidine to prelabel DNA in their liver. Following four weeks of recovery, the rats were placed on either basal diets or diets containing either 0.05% 3'-methyl-4-dimethylaminoazobenzene (3'-MeDAB), 0.028% 2-acetylaminofluorene, or 0.05% 2-methyl-4-dimethylaminoazobenzene for various periods. After five weeks, 3'-MeDAB had caused a dose-dependent loss of prelabeled DNA demonstrating the cytotoxicity of this carcinogen. The comparatively noncarcinogenic 2-methyl-4-dimethylaminoazobenzene caused only a small loss of prelabeled DNA. In contrast, the hepatocarcinogen 2-acetylaminofluorene did not cause a loss of prelabeled DNA, demonstrating its low cytotoxicity. Autoradiography and histology revealed that the loss of prelabeled DNA in livers of rats fed 3'-MeDAB was largely due to parenchymal cell death. Experiments designed to separate liver regenerative hyperplasia from neoplastic hyperplasia revealed the presence of both an early and a delayed elevation of thymidine incorporation into liver DNA in rats fed 0.05% 3'-MeDAB. An "early" elevation of incorporation occurred during and shortly after 3'-MeDAB feeding, and a "delayed" elevation of incorporation occurred some weeks after the dye was discontinued. Autoradiography revealed that parenchymal cells were largely responsible for the increased incorporation. Feeding of 2'-methyl-4-dimethylaminoazobenzene depressed thymidine incorporation. A direct comparison of the effects of isomolar levels of 3'-MeDAB and 2-acetylaminofluorene on hepatic hyperplasia indicated that both carcinogens caused comparable increases in thymidine incorporation, which returned to control levels upon feeding of carcinogen-free diet. The differences and similarities between the responses to the three compounds are discussed and considered with regard to initiation and promotion of hepatoma formation.

1864 *N*-HYDROXY-2-ACETYLAMINOFLUORENE INHIBITION OF RAT LIVER RNA POLYMERASES. (Eng.)
Herzog, J. (Temple Univ. Sch. of Medicine, Philadelphia, Pa. 19140); Serroni, A.; Briesmeister, B. A.; Farber, J. L. *Cancer Res.* 35(8):2138-2144; 1975.

The inhibitory effect of *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF) on hepatic RNA synthesis was investigated in Wistar rats. The rats were fasted overnight and given N-OH-AAF (80 mg/kg, ip). Nuclei isolated two hours after injection showed 50-60% inhibition of RNA synthesis. Chromatin was isolated from the nuclei, and endogneous polymerase activity and template function was assayed. The decreased RNA synthesis was accounted for by an inhibition of the RNA polymerase activities quantitatively solubilized and partially purified from these nuclei. Both nucleolar and nucleoplasmic polymerases were affected. A similar inhibition of the polymerases was demonstrated in intact nuclei by inactivating the endogenous template with actinomycin D and assaying the polymerases with an added exogenous template, poly(deoxyadenylate-deoxythymidylate). Chromatin was prepared from similar nuclear preparations by two methods, differing in the extent to which they remove endogenous polymerase activity. Each chromatin preparation was transcribed with added *Escherichia coli* or partially purified rat liver nucleoplasmic RNA polymerase, respectively. With either polymerase and either chromatin preparation, no inhibition of the template activity of chromatin isolated from H-OH-AAF-treated animals could be detected. It is concluded that N-OH-AAF is a potent inhibitor of rat liver nuclear RNA synthesis and that the mechanism of this inhibition is inactivation of the RNA polymerases. At the same time, N-OH-AAF leaves the chromatin template, at least quantitatively, intact for the synthesis of RNA. The inhibited polymerases may have an altered template specificity allowing for transcription from previously repressed genes. Alternatively, inhibition of the polymerase function, without apparent quantitative effect on the chromatin template, would reduce the level of genetic transcription without interfering with the integrity of the information. This could result in fewer repressors being made, and in some genes being derepressed.

1865 *IN VITRO* BINDING OF *N*-ACETOXY-*N*-2-ACETYLAMINOFLUORENE TO DNA IN CHROMATIN. (Eng.)
Metzger, G. (Institut de Biolgoie Moleculaire et Cellulaire, Laboratoire de Biophysique, 15, rue Descartes, 67000 Strasbourg, France); Daune, M. P. *Cancer Res.* 35(10):2738-2742; 1975.

The binding of *N*-acetoxy-*N*-2-acetylaminofluorene to DNA in native and partially dehistonized chicken RBC chromatin was studied. The amounts of carcinogen bound to DNA were measured, after removal of proteins with phenol, by using the absorption ratio A_{305}/A_{260} or by counting the radioactivity of ^{14}C-labeled carcinogen. Measurements of uncovered zones of DNA in chromatin were made by comparison of results obtained with free DNA and with chromatin at increasing ratios of carcinogen to nucleotide. The proportion of DNA accessible to the carcinogen was found to be 15% in native chicken RBC chromatin and about 22% in native calf thymus chromatin. The amount of accessible DNA increased to 55% in chicken RBC chromatin depleted of histones H1 and H5. Formaldehyde unwinding performed on DNA extracted from chromatin after modification

showed an increasing number of defects in the double helix with the amount of DNA-fixed carcinogen. At high ratios of carcinogen to nucleotide, the recoveries of DNA (by phenol method) and of histones (by acidic extraction) decreased with increasing ratios. This suggests a covalent linkage between proteins and DNA.

1866 METABOLISM AND DISPOSITION OF N-{4-(5-NITRO-2-FURYL)-[2-^{14}C]THIAZOLYL}ACETAMIDE IN THE RAT. (Eng.) Wang, C. Y. (Univ. Wisconsin Med. Sch., Madison); Chiu, C. W.; Bryan, G. T. *Drug Metab. Dispos.* 3(2):89-95; 1975.

The metabolism and disposition of ^{14}C-labeled N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (^{14}C-NFTA) in Sprague-Dawley rats was investigated. Dimethylformamide (0.2 ml) containing 1.46×10^6 dpm ^{14}C-NFTA administered i.p. to young (50 g) and lactating (300 g) female rats was rapidly absorbed through the peritoneum and deposited in the urine, intestinal contents and feces. Less than 0.5% of the radioactivity was expired as CO_2 during the first 24 hr. Two yellow metabolites, accounting for 5 and 27% of the radioactivity in urine, were found by paper chromatography. One metabolite retained the 2-amino-4-(5-nitro-2-furyl)thiazole moiety. Less than 0.5% of the radioactivity in the urine was due to unchanged ^{14}C-NFTA. The radioactivity level in the visceral organs reached a maximum 2 hr after dosing and then gradually decreased. Liver contained considerably more radioactivity (80% after 24 hr) than did other visceral organs; this was mainly distributed in the cytosol and 900 *g* pellets. Fifty percent of the radioactivity in the liver was bound to the hot trichloroacetic acid (TCA)-insoluble fraction; that bound to the cold TCA-insoluble fraction increased gradually from 66.9% at 2 hr to 82.6% at 24 hr. The radioactivity bound to the cold and hot TCA-insoluble fractions in kidney remained at 30% and 20%, resp. Nitroreduction of ^{14}C-NFTA by microsomes, as a prerequisite of binding of metabolite to microsomal protein, was demonstrated. Addition of L-cysteine or reduced glutathione to the incubation mixture did not alter the nitroreductase activity of the microsomes; however, binding of radioactivity to protein was significantly decreased. Addition of other amino acids did not significantly decrease the nitroreductase activity or binding to protein. These results suggest that reduced NFTA binds to protein sulfhydryl groups. Since ^{14}C-NFTA binds to macromolecules *in vivo*, nitroreduction may be important for the metabolism of 5-nitrofurans.

1867 ADDUCTS BETWEEN THE CARCINOGEN 2-ACETAMIDOPHENANTHRENE AND ADENINE AND GUANINE OF DNA. (Eng.) Scribner, J. D. (Fred Hutchinson Cancer Res. Cent., Seattle, Wash.); Naimy, N. K. *Cancer Res.* 35(6):1416-1421; 1975.

The involvement of the carcinogen *N*-acetoxy-2-acetaminidophenanthrene (N-AcO-AAP) with nucleosides of DNA was investigated. Tritiated N-AcO-AAP was added to calf thymus DNA and incubated for two days. Extracted DNA was then incubated with unlabeled deoxyquanosine acetamidophenanthrene (dG-AAP) and with deoxyadenosine acetamidophenanthrene (dA-AAP) for 42 hr, applied to a Sephadex LH-20 column, and eluted with 35% ethanol. The eluted DNA was isolated and degraded and reapplied to the column. The two peaks eluting corresponded to dG-AAP and DG-AAP. Combining the two eluates, the mixture was analyzed by thin layer chromatography. Counts were found coincident with dA-AAP (retardation factor, 0.60) and dG-AAP (retardation factor 0.73), but 75% of the counts migrated with the front. This large entity was not characterized. These results indicate that there is no typical reaction between nucleic acids and esters of *N*-arylacetohydroxamic acids. It is suggested that the transacetylation proceeds at the same rate for all *N*,*O*-diacetyl-*N*-arylhydroxylamines. The difference in yields of purine-*N*-arylacetamide adduct between 2-acetamidofluorene and *N*-acetoxy-4-acetamidobiphenyl lies in the relative rates of reaction. The target purine will be determined by the preferred reaction site on the nitrenium ion (i.e. reacting at nitrogen the target would be C-8 of guanine, reaction at carbon-target would be N-6 of adenine). The *N*-arylnitrenium ions will have a greater preference for the reaction at nitrogen giving a precursor preference for guanine. The results could substantiate this rule, but further testing is necessary.

1868 SEQUENTIAL HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE RAT LIVER AFTER SINGLE-DOSE AFLATOXIN B_1 INTOXICATION. (Eng.) Kalengayi, M. M. R. (Laboratorium voor Histochemie en Cytochemie, Departement Medische Navorsing, Fakulteit Geneeskunde, Katholieke Universiteit Leuven, B 3000 Leuven, Belgium); Desmet, V. J. *Cancer Res.* 35(10):2836-2844; 1975.

Sequential histological and histochemical changes in the liver of male Wistar rats were followed from six hours to 17 mo after administration of an LD_{50} dose of aflatoxin B_1 (7.20 mg/kg) in food. Periportal liver cell necrosis and marked biliary cell proliferation were observed at three and six days. Periportal cytoplasmic glycogen and RNA depletion occurred during this early period and subsequently extended to the whole lobule. Activities of alkaline phosphatase, 5-nucleotidase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, ATPase, succinic dehydrogenase, and alkaline nuclease decreased or disappeared in the periportal area. While alkaline phosphatase increased strikingly in the centrolobular area, canalicular ATPase completely disappeared throughout the liver lobule. The histochemical changes reverted to normal after cessation of the necrosis. Histochemical techniques were more sensitive in detecting the vulnerability of the periportal parenchyma to aflatoxin B_1. After the necrosis, regenerative foci appeared from day 11 to 32. These showed a variable content of glycogen and RNA and were characteristically enzyme-deficient. This reflects the immaturity of regenerating hepatocytes. These early foci subsequently disappeared and are thus considered irrelevant to hepatocarcinogenesis. In contrast, the enzyme-deficient foci that appeared

at 17 mo may represent insidious sites of carcinogenesis. These foci strongly resembled the preneoplastic enzyme-deficient areas observed in a long-term hepatocarcinogenesis study; moreover, it has been shown that liver cell carcinomas may develop after 18-26 mo in rats given a single LD_{50} of aflatoxin B_1.

1869 THE RESPONSE OF *XERODERMA PIGMENTOSUM* CELLS AND CONTROLS TO THE ACTIVATED MYCOTOXINS, AFLATOXINS AND STERIGMATOCYSTIN. (Eng.) Stich, H. F. (Cancer Res. Centre, Univ. British Columbia, Vancouver, Canada); Laishes, B. A. *Int. J. Cancer* 16(2):266-274; 1975.

The activation of the mycotoxins aflatoxin B_1, G_1, B_2, G_2, aflatoxicol, and sterigmatocystin by microsomal and supernatant fractions of livers of several species (Syrian hamster, Swiss mouse, New Zealand white rabbit, Muscovite duck, and Rainbow trout) was examined. Cultured fibroblasts of normal persons and of DNA repair-deficient *Xeroderma pigmentosum* patients were then incubated with these treated fractions to compare their effects. A 9S fraction microsomal preparation of liver (105,000 x *g*) or 105,000 x *g* supernatant of liver was cultured with the fibroblasts. DNA repair synthesis, chromosome aberrations and clone forming capacity were used as endpoints. The activation mixtures significantly increased the chromosome breaking function, lethality and DNA damaging effect (measured as DNA repair synthesis) of aflatoxin B_1, G_1, aflatoxicol, and sterigmatocystin. The DNA repair-deficient XP cells responded to the activated mycotoxins with a low level of unscheduled ^{3}HTdR incorporation as compared to that of control cells, but showed a highly elevated sensitivity to the chromosome-damaging and lethal effect of aflatoxin B_1 and sterigmatocystin. The results emphasize the importance of employing activation procedures and of using "sensitive" human cells to reveal the cytotoxic effects of chemical compounds.

1870 CHOLERA TOXIN EFFECTS ON CELL GROWTH ACCOMPANIED BY SELECTIVE ALTERATIONS IN METABOLITE UPTAKE AND MODIFICATION OF CELL SURFACE PROTEINS. (Eng.) Rieber, M. (Center of Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 101, Venezuela); Bacalao, J.; Alonso, G. *Cancer Res.* 35(11):3009-3013; 1975.

Exposure of Chinese hamster ovary cells to cholera toxin at 1 or 0.1 μg/ml caused the cells to become increasingly elongated and flattened, and increased the adherence of the cells to the surface of the petri dishes in which they were seeded. These toxin-mediated morphological changes were paralleled by alterations in surface proteins detectable by lactoperoxidase-catalyzed radioiodination of outer proteins. Mild trypsin (1 μg/ml) treatment of cells prelabeled with ^{3}H-glucosamine (20 μCi) revealed that toxin-treated cells exposed more trypsin-sensitive glycoproteins than did rounded control cells. An alteration in a specific glycoprotein species became evident by polyacrylamide gel electrophoresis followed by fluorography of ^{3}H-labeled cellular glycoproteins. A slow-migrating glycoprotein with an apparent molecular wt of 180,000 was present in control but not in toxin-treated cells. Mild tryptic treatment of the control cells removed this glycosylated component, giving a profile essentially identical to that of toxin-exposed cells. The effects of cholera toxin on surface proteins and cell growth occurred in the absence of a modification in leucine uptake or incorporation of leucine into protein. However, cholera toxin decreased the incorporation of ^{3}H-thymidine into DNA and increased glucosamine incorporation into acid-soluble, lipid-soluble, and alkali-soluble fractions. Colchicine (10^{-7} M) antagonized the morphological effects of cholera toxin but not the effects of toxin on glucosamine utilization.

1871 HETEROCHROMATIN AND CHROMOSOME ABERRATIONS: A COMPARATIVE STUDY OF NORMAL AND TUMOUR CELLS OF MOUSE WITH VARIOUS DISTRIBUTIONS OF HETEROCHROMATIN. (Eng.) Natarajan, A. T. (Dept. of Radiation Biology, Wallenberglaboratoriet, Stockholm Univ., Stockholm, Sweden); Raposa, T. *Mutat. Res.* 29(2):199-200; 1975.

A comparative study was made of mitomycin-C-induced chromosome aberrations in three types of mouse cells differing in chromosome number and karyotype. The materials studied are listed with their individual characteristics: (1) Laboratory mouse (2n=40) had all telocentric chromosomes possessing heterochromatin near the centromere (except Y). (2) The F_1 hybrid (2n=33) of laboratory mouse x tobacco mouse had seven metacentrics and 26 telocentrics. Its heterochromatin was located near the centromeres in all chromosomes. (3) Mouse ascites tumor cells (2n=28-29) had 9-10 biarmed chromosomes. The heterochromatin was located in the centromeric regions in all except two marker chromosomes, one of which was telocentric with four blocks of heterochromatin distributed along the length, and the other was a biarmed chromosome with two blocks of heterochromatin located at the distal part of the long arm. The tumor cells were treated *in vivo*, and the other two materials were treated *in vitro* with different doses of mitomycin C. The types of aberration scored included all chromatic intrachanges and interchanges. In the laboratory mouse, about 80% of the aberrations (of which 40% were intrachanges) were located in the heterochromatic regions. In the tobacco mouse x laboratory mouse hybrid, the exchanges were between two telocentrics or telocentric and metacentric, but seldom between two metacentrics, indicating a restriction on the association between two metacentrics in the interphase. In the tumor cells, the centrometric heterochromatin was involved more often in interchanges (about 95%) than in intercalary blocks. Intrachanges involving the marker telocentric were common, and there was no restriction between two biarmed chromosomes to form exchanges. The overall frequency of intrachanges was very low in this cell line.

1872 A COMPARATIVE STUDY OF THE EFFECTS OF LUCANTHONE (MIRACIL D) AND ACTINOMYCIN D ON THE CHINESE HAMSTER CELLS GROWN IN CULTURES. (Eng.) Epifanova, O. I. (Inst. Molecular Biology, U.S.S.R. Acad. Sciences, Moscow B-312, U.S.S.R.); Makarova, G. F.; Abuladze, M. K. *J. Cell. Physiol.* 86(2/Suppl. 1/Part I):261-268; 1975.

Chinese hamster cells of an established clone line grown in monolayers were incubated for up to two hours with either lucanthone (0.3-30 μg/ml) or actinomycin D (0.06-0.10 μg/ml) and subjected to radioautographic investigations with ^{3}H-uridine during the period of treatment. At concentration of 9 μg/ml, lucanthone selectively inhibited the synthesis of nucleolar (ribosomal) RNA while the extranucleolar RNA synthesis proceeded at a high level. Similar results were obtained with 0.08 μg/ml actinomycin D. Protein synthesis and mitotic activity were also affected by lucanthone but the drug did not markedly interfere with DNA synthesis. Lucanthone appeared to be much less effective in cell killing than actinomycin D and its inhibitory effects on the nucleolar RNA synthesis and other cellular processes proved readily reversible. The results indicate that lucanthone may be useful as a tool for studying RNA synthesis in animal cells.

1873 INDUCTION BY ALKYLATING AGENTS OF SISTER CHROMATID EXCHANGES AND CHROMATID BREAKS IN FANCONI'S ANEMIA. (Eng.) Latt, S. A. (Harvard Medical Sch., Boston, Mass. 02115); Stetten, G.; Juergens, L. A.; Buchanan, G. R.; Gerald, P. S. *Proc. Natl. Acad. Sci. USA.* 72(10):4066-4070; 1975.

Sister chromatid exchanges, which may reflect chromosome repair in response to certain types of DNA damage, provided means of investigating the increased chromosome fragility characteristic of Fanconi's anemia. Sister chromatid exchanges were detected by fluorescence microscopy in phytohemagglutinin-stimulated lymphocytes from male patients with Fanconi's anemia after culturing with 2.5 x 10^{-5} M bromodeoxyuridine and staining with 33258 Hoechst. The baseline frequency of these sister chromatid exchanges differed little from those of normal lymphocytes. However, addition of mitomycin C (0.01 or 0.03 μg/ml) to the Fanconi's anemia cells during culture induced less than half of the increase in exchanges found in identically treated normal lymphocytes. This reduced increment in exchanges was accompanied by a partial suppression of mitosis and a marked increase in chromatid breaks and rearrangements. Many of these events occurred at sites of incomplete chromatid interchange. The increase in sister chromatid exchanges induced in Fanconi's anemia lymphocytes by ethylmethane sulfonate (0.25 mg/ml) was slightly less than that in normal cells. Lymphocytes from two sets of parents of the patients with Fanconi's anemia exhibited a normal response to the two alkylating agents. Dermal fibroblasts from two different patients with Fanconi's anemia reacted to mitomycin C with an increase in chromatid breaks, but a nearly normal increment of sister chromatid exchanges. The results suggest that chromosomal breaks and rearrangements in Fanconi's anemia lymphocytes may result from a defect in a form of repair of DNA damage.

1874 COLON CARCINOGENESIS WITH AZOXYMETHANE AND DIMETHYLHYDRAZINE IN GERM-FREE RATS. (Eng.) Reddy, B. S. (Naylor Dana Inst. Dis. Prev., New York, N.Y.); Narisawa, T.; Wright, P.; Vukusich, D.; Weisburger, J. H.; Wynder, E. L. *Cancer Res.* 35(2):287-290; 1975.

The effect of intestinal microflora on the sensitivity of the colon to the carcinogenic effect of azoxymethane and a large dose of 1,2-dimethylhydrazine was studied using germ-free and conventional female Fischer rats. Injection sc of 1,2-dimethylhydrazine (20 mg/kg/wk for 20 wk) induced tumors of the ear duct, kidney, and small intestine of conventional rats but none in germ-free animals. Only 20% of 24 germ-free rats showed 1,2-dimethylhydrazine-induced colonic tumors, whereas 93% of 15 conventional rats developed multiple colonic tumors. Twenty weekly intrarectal instillations of azoxymethane (10 mg/kg/wk) appreciably increased the multiplicity of colonic tumors in germ-free rats and in gnotobiotic rats contaminated with *Clostridium perfringens*, as compared to conventional controls. None of the germ-free rats showed ear duct tumors. The incidence of kidney tumors was lower in germ-free rats than in other groups. It is concluded that the intestinal microbial populations alter the effect of carcinogens in the large intestine.

1875 THE REDUCTION OF *N*-HYDROXY-4-ACETYLAMINOBIPHENYL BY THE INTESTINAL MICROFLORA OF THE RAT. (Eng.) Wheeler, L. A. (Beth Israel Hosp., Boston, Mass. 02215); Soderberg, F. B.; Goldman, P. *Cancer Res.* 35(11/Part 1):2962-2968; 1975.

The role of the intestinal flora in the conversion of *N*-hydroxy-4-acetyl-aminobiphenyl (N-OH-AABP) to 4-acetylaminobiphenyl was examined. This reaction, which reverses the metabolic activation of the parent carcinogen, could be demonstrated in 18-hr cultures of some bacteria indigenous to the intestinal micorflora to which 50 μg/ml N-OH-AABP had been added. These included cultures of *Clostridium* sp., *Clostridium perfringens*, *Peptostreptococcus productus* I, and *Bacteroides fragilis* ss. *thetaiotaomicron* and ss. *vulgatus*. In contrast, cultures of *Lactobacillus plantarum* and *Escherichia coli* showed little or no capacity for this reaction. The reduction of N-OH-AABP was also carried out by homogenates of liver, kidney, and brain of Sprague-Dawley rats. On a weight basis, the cecal flora were considerably more active in reducing N-OH-AABP than were homogenates of tissues of the gastrointestinal tract. The cecal flora also had a greater activity for reducing N-OH-AABP than the stomach flora, an observation which may relate to the induction of tumors in the forestomach but not in the cecum of rats fed this compound. The products of the metabolism of N-OH-

AABP were compared in germ-free and conventional animals. Glucuronide conjugates of N-OH-AABP were found in the cecal contents and feces only of the germ-free rats, while 4-acetylaminobiphenyl was found in the feces only of conventional rats. These results suggest that the flora, by hydrolyzing glucuronides and reducing N-OH-AABP, may influence the level of metabolites of 4-acetylaminobiphenyl, which are critical for carcinogenesis.

1876 FLUORESCENCE SPECTRAL EVIDENCE THAT BENZO(*a*)PYRENE-DNA PRODUCTS IN MOUSE SKIN ARISE FROM DIOL-EPOXIDES. (Eng.) Daudel, P. (Institut du Radium, 11, Rue Pierre et Marie Curie, 75231 Paris, Cedex 05, France); Duquesne, M.; Vigny, P.; Grover, P. L.; Sims, P. *FEBS Lett.* 57(3):250-253; 1975.

A photon-counting spectrofluorometer was used to compare the fluorescence spectral characteristics of DNA from benzo(*a*)pyrene-treated mouse skin with those of salmon sperm DNA treated in solution with reactive benzo(*a*)pyrene derivatives. DNA was isolated from the skin of 15 male C57Bl mice treated on the back with benzo(*a*)pyrene (250 μg) in acetone (0.15 ml). Salmon sperm DNA (10 mg) was reacted with one of the following benzo(*a*)pyrene epoxides (200 μg) in acetone (5 ml): 7,8-dihydro-7,8-dihydroxybenz(*a*)pyrene 9,10-oxide; 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene 7,8-oxide; and benzo(*a*)-pyrene 4,5-oxide. The main fluorescence characteristics of DNA from hydrocarbon-treated mouse skin closely resembled those of salmon sperm DNA treated with the 7,8-diol 9,10-epoxide. The spectra show that the main benzo(*a*)pyrene derivative bound to the DNA of mouse skin retains an intact pyrene nucleus. This indicates that metabolism of the 7,8,9,10-ring precedes reaction with DNA and is consistent with the hypothesis that a diol-epoxide formed on this ring is involved. The major metabolite could not be further characterized, because both isomeric benzo(*a*)pyrene diol-epoxides gave similar fluorescence spectra after reaction with DNA. Studies on DNA treated with benzo(*a*)pyrene 4,5-oxide confirmed that DNA products formed by the *in vivo* metabolization of benzo(*a*)pyrene do not result from reaction with K-region epoxides. The fluorescence maxima for the K-region epoxide-reacted DNA were clearly different from those of benzo(*a*)pyrene-treated mouse skin DNA and of DNA reacted with diol-epoxides. These results, as well as those previously reported for 7-methyl-benz(*a*)anthracene, demonstrate the high sensitivity of the photon-counting spectrofluorometer.

1877 ENZYMIC FORMATION OF 6-OXOBENZO[*a*]PYRENE RADICAL IN RAT LIVER HOMOGENATES FROM CARCINOGENIC BENZO[*a*]PYRENE. (Eng.) Lesko, S. (Johns Hopkins Univ., Baltimore, Md. 21205); Caspary, W.; Lorentzen, R.; Ts'o, P. O. P. *Biochemistry* 14(18):3978-3984; 1975.

Upon incubation of benzo(*a*)pyrene [B(*a*)P] at 37 C in rat liver homogenates fortified with NADPH-generating cofactors, a metabolite was formed which gave rise spontaneously to an electron paramagnetic resonance (EPR) signal. The radical and its metabolic precursor were extracted into benzene, in which they were relatively stable, for measurement. The EPR signal was identical to that extracted after incubating synthetic 6-hydroxybenzo(*a*)pyrene [6-OH-B(*a*)P] in rat liver homogenates and was identified as the 6-oxobenzo(*a*)pyrene radical by its characteristic hyperfine structure. No EPR signal was detected when benzo(*a*)pyrene or cofactors were eliminated from the incubation mixture or when the homogenate was heated. The concentration of radical peaked after 14-15 min of incubation and declined rapidly to a low level at 20 min. The decay of 6-OH-B(*a*)P in rat liver homogenate did not appear to be enzymic, and showed a first-order rate constant of 0.29/min and a half-life of 2.4 min. The only significant products formed by the oxidation of 6-OH-B(*a*)P in rat liver homogenates were 6,12-benzo(*a*)pyrene dione (15%), 1,6-benzo(*a*)pyrene dione (41%), and 3,6-benzo(*a*)pyrene dione (44%). Formation of 6-OH-B(*a*)P represented about 18% and 20% of total B(*a*)P metabolism for female Sprague-Dawley rats and female ACI rats, respectively. The data indicate that 6-OH-B(*a*)P is a major metabolite of B(*a*)P. This metabolite is very labile because it is readily oxidized to the 6-oxobenzo(*a*)pyrene radical, which is a transient intermediate in the further oxidation to quinones.

1878 TRANSPLACENTAL AND DIRECT ACTION OF BENZO(a)PYRENE STUDIED IN ORGAN CULTURES OF EMBRYONIC LUNG TISSUE. (Eng.) Shabad, L. M. (Acad. Medical Sciences USSR, 115 478 Moscow, USSR); Kolesnichenko, T. S.; Nikonova, T. V. *Neoplasma* 22(2):113-122; 1975.

Transplacental effects of benzo(a)pyrene (BP) were studied in organ cultures of embryonic lung tissue explanted from mouse donors injected with the carcinogen. BP and pyrene were injected into female A and C57BL strain mice at 14-15 days of gestation. Lung tissue of 19-20-day-old embryos was explanted to organ culture. Of 551 cultures from strain A mice treated with 4 mg BP, 47.17% showed epithelial hyperplasia; at 12 mg BP, 36.64% of 1,146 cultures showed hyperplasia, and 41.01% showed adenomatous change. Of 708 cultures from C57BL mice treated with 4 mg BP, 15.39% exhibited hyperplasia; 25.67% of 1,098 cultures of mice treated with 12 mg BP showed hyperplasia. To study the direct action of BP, 6 μg/ml was added to medium containing explants from embryos of untreated mice. Of 357 strain A explants and 568 strain C57BL explants, 8.9% and 2.1%, respectively showed hyperplasia. No alterations were seen in cultures in which the mice had been injected with pyrene. The results confirm the possibility of transplacental blastomogenic activity of BP. This activity varies with the strain of mouse used. Direct application of BP or pyrene does not cause blastomogenic activity. A growth-promoting effect of benzo(a)pyrene was also noted.

1879 PRODUCTION OF RESPIRATORY TRACT TUMORS IN HAMSTERS BY BENZO[*a*]PYRENE. (Eng.)

Kobayashi, N. (Inst. Pulmonary Cancer Res., Chiba Univ., Inohana 1-8-1, Chiba 280, Japan). *Gann* 66(3):311-315; 1975.

The tumorigenic activity of benzo[*a*]pyrene without a surface-active agent or carrier dust was tested. A group of 32 male and 28 female Syrian golden hamsters was given weekly intratracheal instillation of 1 mg of benzo[*a*]pyrene suspended in isotonic saline, for 30 wk. Squamous cell carcinoma, adenocarcinoma, anaplastic carcinoma, adenoma, papilloma, and polyps were induced in the respiratory tracts of the animals. Tumor incidences were 42.3% in males and 57.7% in females. Respiratory carcinomas were inducible in hamsters by simple instillation of a low dose of benzo[*a*]pyrene without using a surface-active agent or carrier-dust. These findings may be useful as a standard data for cocarcinogenesis studies when using another modality combined with benzo[*a*]pyrene.

1880 METABOLISM OF BENZO[a]PYRENE: EFFECT OF SUBSTRATE CONCENTRATION AND 3-METHYLCHOLANTHRENE PRETREATMENT ON HEPATIC METABOLISM BY MICROSOMES FROM RATS AND MICE. (Eng.) Holder, G. M. (Natl. Inst. Health, Bethesda, Md. 20014); Yagi, H.; Jerina, D. M.; Levin, W.; Lu, A. Y. H.; Conney, A. H. *Arch. Biochem. Biophys.* 170(2):557-566; 1975.

The metabolism of [^{14}C]benzo[*a*]pyrene by liver microsomes from normal and 3-methylcholanthrene (3-MC)-treated mice and rats was quantitatively analyzed by high-pressure liquid chromatography. Genetically responsive (3-MC inducible) C57BL/6J mice, and genetically unresponsive DBA/2J mice, (aged 7-8 wk) and adult male Long-Evans rats (aged ten weeks) were injected ip with 25 mg/kg 3-MC in corn oil once daily for three days. The ratio of dihydrodiols of benzo[*a*]pyrene to total metabolites formed was greater with microsomes from Long-Evans rats when compared with the ratio produced by microsomes from both strains of mice. Treatment of rats or C57BL/6J mice with 3-MC resulted in marked increases in the metabolism of benzo[*a*]pyrene to phenols, dihydrodiol and quinones; this effect did not occur in DBA/2J mice. The profile of benzo[*a*]pyrene metabolites formed by rat and mouse liver microsomes was dependent on both substrate and protein concentration. The use of incubation conditions with limiting substrate concentrations resulted in massive secondary metabolism of benzo[*a*]pyrene metabolites; this was accompanied by the production of substantial amounts of metabolites that were no longer extractable into the organic phase.

1881 ARGYROPHILIC CARCINOIDS IN TWO SYRIAN HAMSTERS (*MESOCRICETUS AURATUS*). (Eng.) Dunham, L. J. (Natl. Cancer Inst., Bethesda, Md.); Snell, K. C.; Stewart, H. L. *J. Natl. Cancer. Inst.* 54(2):507-513; 1975.

Carcinoid tumors developing in the gastrointestinal tract of two Syrian hamsters (*Mesocricetus auratus*) fed a diet containing arecoline (0.1%) plus calcium hydroxide (2.5%) or an aromatic snuff (2.5%) plus calcium hydroxide (2.5%) for five days/week were studied microscopically and described. The hamster developing carcinoid of the glandular stomach was fed the arecoline-supplemented diet for 12 months; the other, which developed pancreatic carcinoid, was fed the snuff-supplemented diet for 16 months. The carcinoid of the glandular stomach was tan-colored and indurated, and was located on the greater curvature of the stomach. Focal multicentric proliferation was noted in the gastric mucosa with penetration into the submucosa. Argyrophilic granules were present. The pancreatic carcinoid was firm, gray-white in color and irregularly shaped. There was a connective tissue capsule with extension into mesenteric fat and invasion into local veins and lymphatics. Cells were grouped in a focal arrangement and argyrophilic granules were noted. It was not determined whether the development of these carcinoids was related to treatment.

1882 CHROMOSOME ANALYSIS OF LYMPHOCYTES FROM CADMIUM WORKERS AND ITAI-ITAI PATIENTS. (Eng.) Bui, T. H. (Karolinska Hosp., Stockholm, Sweden); Lindsten, J.; Nordberg, G. F. *Environ. Res.* 9(2):187-195; 1975.

Chromosome analysis was made on cultured lymphocytes from five cadmium-exposed Swedish workers, four Japanese Itai-itai patients, and seven Japanese and Swedish control subjects to determine whether cadmium exposure might result in an increased frequency of chromosome aberrations. Cadmium levels in whole blood was determined by flameless atomic absorption spectrophotometry and in urine by atomic absorption spectrophotometry. Chromosome analysis was performed on cells from conventional lymphocyte macrocultures using the subjects' own plasma. Chromosome preparations were made by the air-drying technique and stained with Giemsa. All slides from exposed as well as control subjects were coded, mixed and analyzed blindly in both materials. Whenever possible, 100 consecutive metaphases were analyzed in detail for chromosome abnormalities. The blood levels of cadmium, which mainly reflect exposure during the previous months, were consistently higher in the Itai-itai patients and in the cadmium exposed workers than in the corresponding control subjects. The urinary values of cadmium displayed a similar pattern. The mean value (20.0 μg/g creatinine) for the Itai-itai patients, however, was higher than that (11.5 μg/g creatinine) for the cadmium workers. This may reflect a higher burden of cadmium and a more pronounced renal tubular damage in the Itai-itai patients than in the cadmium workers. There was no statistical difference between the patients and the Japanese control subjects (6.6 and 6.0%, respectively) or between the Swedish cadmium-exposed workers and the Swedish controls (2.0 and 4.7%, respectively) with regard to the frequency of cells with chromosome breakage. The Japanese subjects, however, demonstrated a significantly higher frequency of chromosomally abnormal cells than the cadmium-exposed Swedish workers and control subjects. In contrast to previous reports, no evidence was ob-

tained to indicate that cadmium induces chromosome damage *in vivo* in man; some other agent(s) to which the cells from both the Japanese patients and control subjects had been exposed must have caused the increase in chromosome aberrations. This increase only included chromosome breaks and not chromatid breaks, pointing to an effect prior to the phase of DNA synthesis of the cell cycle.

1883 DIVERSE MECHANISMS OF HEPATOCELLULAR INJURIES DUE TO CHEMICALS: EVIDENCE IN RATS ADMINISTERED CARBON TETRACHLORIDE OR DIMETHYLNITROSAMINE. (Eng.) Nayak, N. C. (Dep. Pathol., All India Inst. Med. Sci., New Delhi); Chopra, P.; Dhar, A.; Das, P. K. *Br. J. Exp. Pathol.* 56(2):103-112; 1975.

Acute hepatotoxicity of CCl_4 (100-500 μl/100 gm) and dimethylnitrosamine (DMN, 5-50 mg/kg) in normal and phenobarbitone-treated fetal, newborn and adult rats was investigated. The accumulation of intracellular lipid and necrosis of centrizonal hepatocytes was dose-dependent. Pretreated and untreated fetuses and newborns showed no ill effects of CCl_4 at exposures of 24 or 48 hr. All animals pretreated with phenobarbitone showed excess hepatic lipid, regardless of whether they were treated with CCl_4. The livers of newborn animals pretreated with phenobarbitone showed diffuse balloon cells, focal or centrazonal areas of necrosis, and degenerating cells clumping eosinophilic cytoplasm; necrobiotic cells resembling Councilman bodies were also seen in varying combinations. CCl_4 in pretreated adults induced excessive lesions compared to controls. Partially hepatectomized animals treated with nonlethal and lethal doses showed less severe alterations. DMN (15 mg) in adults caused only mild centrizonal congestion, but no necrosis or steatosis. A 30 mg dose of DMN caused intense congestion, hemorrhage and severe necrosis of the hemorrhagic or the congested type. Normal fetuses and newborns showed similar conditions. Pretreatment with phenobarbitone produced only mild congestion without necrosis when DMN was administered. Hepatectomy increased the toxicity of DMN by greater than 50%. Phenobarbitone did increase liver triglycerides, but the effect of CCl_4 was not additive. Electron microscopic study of hepatocytes of pretreated fetuses revealed the cells to be larger, having increased lipid but less smooth endoplasmic reticulum. Similar examination of the hepatocytes of newborns showed an increase in smooth endoplasmic reticulum. The distinct differences in the effect of these two chemicals in varying conditions most likely involves changes at the molecular level.

1884 EFFECT OF DIMETHYLNITROSAMINE ON PERSISTENCE OF METHYLATED GUANINES IN RAT LIVER AND KIDNEY DNA. (Eng.) Nicoll, J. W. (Middlesex Hosp. Med. Sch., London, England); Swann, P. F.; Pegg, A. E. *Nature* 254(5497):261-262; 1975.

The persistence of O^6-methylguanine and 7-methylguanine was studied in the DNA of rat liver and kidney following the administration of a large dose of dimethylnitrosamine (20 mg/kg, which would be expected to induce some kidney tumors), and following a nontumorogenic dose of 2.5 mg/kg. The 7-methylguanine in both liver and kidney DNA had a half-life of about 60 hr after the large dose of dimethylnitrosamine, following the smaller dose, the rate of loss was about the same in kidney DNA and slightly slower in liver. The O^6-methylguanine of the DNA of rat liver and kidney was only about 10% of the amount of 7-methylguanine. The product was lost from liver DNA after either dose with a half life of about 20 hr, and after the low dose of dimethylnitrosamine it was lost from kidney DNA at a slightly lower rate. After the high dose, there was an initial drop of about 30% in the O^6-methylguanine of kidney DNA; no change occurred from 15-50 hr, and a slow rate of loss was observed after 50 hr. The potentially mutagenic product is longer-lived than in liver DNA after either dose. Carcinogenic susceptibility of various organs may be determined by the ability to repair certain alterations in DNA produced by the carcinogen.

1885 METHYLATION OF RAT LIVER MITOCHONDRIAL DEOXYRIBONUCLEIC ACID BY CHEMICAL CARCINOGENS AND ASSOCIATED ALTERATIONS IN PHYSICAL PROPERTIES. (Eng.) Wilkinson, R. (Middlesex Hosp. Med. Sch., London, England); Hawks, A.; Pegg, A. E. *Chem. Biol. Interact.* 10(3):157-167; 1975.

Alkylation of hepatic mitochondrial and nuclear DNA, as reflected by methylation of guanine to 7-methylguanine, was measured five hr after administration of ^{14}C-dimethylnitrosamine (DMN, 3.4-27 mg/kg, i.p.) and four hr after methyl methanesulfonate (MMS 60-120 mg/kg, i.p.) to Wistar rats. At all doses, DMN was more effective in alkylating mitochondrial DNA than nuclear DNA, but MMS was equally effective on both kinds of DNA. At the highest dose of DMN, 0.72% of guanine was methylated in nuclear DNA compared to 1.21% of mitochondrial DNA guanine; corresponding figures for the highest dose of MMS were 0.09% and 0.10%. Gel electrophoresis and isopycnic centrifugation in CsCl gradients in the presence of ethidium bromide indicated that DMN caused a considerable decrease in the amount of mitochondrial DNA which could be isolated in the closed circular form; at the highest dose of DMN no closed circular mitochondrial DNA was detected. MMS was less effective in reducing the amount of closed circular mitochondrial DNA. DMN may cause strand breaks in mitochondrial DNA, and this system may have possible use in investigating carcinogen-induced breaks in DNA.

1886 REVERSIBILITY OF LYSOSOMAL AND GLUCOSE 6-PHOSPHATASE CHANGES PRODUCED IN THE RAT LIVER BY DIMETHYLNITROSAMINE. (Eng.) Hendy, R. (British Ind. Biol. Res. Assoc., Carshalton, England); Grasso, P. *Chem. Biol. Interact.* 10(6):395-406; 1975.

Tuck Wistar rats were repeatedly injected with various solutions of dimethylnitrosamine (0.2 ml/100 g) in saline to determine whether a cumulative toxic

ffect was produced in the liver. Doses were adminstered sc at 1.0, 0.3, or 0.1 mg/kg/day for 4, 8, r 12 wk. Doses were too low to produce cytopathoogical changes, as indicated by depression of gluose-6-phosphatase and induction of autophagic vaculeles (AV) in hepatocytes, when given once only. Sinle sc injections of 10 or 3 mg/kg induced these cytoathological changes in the centrilobular (CLB) heatic cells, but when the dose was reduced to 1 mg/kg o such changes were seen. After daily administraion of 1 mg/kg for 4 or 8 wk, both glucose-6-phoshatase depression and autophagy were observed, in ddition to marked hypertrophy of the rough endolasmic reticulum, nucleolar microsegregation and he appearance of distorted, often ring-shaped mitohondria with shortened cristae. Kupffer cells exibited a marked increase in lysosomal activity. ith the exception of mitochondrial changes and upffer cell activity this picture was observed, in ilder form, when the dose administered was 0.3 or .1 mg/kg daily for the same period. When treatent was continued for 12 wk, however, the only ifferences from control rats were the presence of ypertrophied rough endoplasmic reticulum (RER) at ll three dose levels, nucleolar microsegregation t the upper two dose levels, and pronounced Kupffer ell activity at the highest dose. These findings ndicate that cumulative cytopathologic effects occur nly up to eight weeks at the dose levels studied, ut hypertrophy of RER and increased Kupffer cell ctivity persist up to 12 wk. The observed nuclelar and ER alterations may be important in the inuction of hepatocellular carcinoma by dimethylnirosamine.

887 INCREASE IN CYSTATHIONINE β-SYNTHASE ACTIVITY IN THE LIVER OF RATS TREATED ITH L-ETHIONINE. (Eng.) Koraćević, D. (Fac. Med., is, Yugoslavia). *Experientia* 31(1):26-27; 1975.

he effect of L-ethionine on cystathionine β-synthse activity was studied in male and female albino ats. L-ethionine, actinomycin D, cycloheximide, nd ATP were injected i.p. in doses of 400 mg, 1 mg, 0 mg, and 200 mg/kg body wt, resp. DL-methionine, -methionine-DL-sulfoximine, and DL-methionine-ulfone were administered by the same route in an mount equimolar to the quantity of ethionine. Rats ere killed 3 hr after ethionine injection. The ingle dose of ethionine caused an approximately ive-fold increase in enzyme activity above normal evels. There was no sex difference. Pretreatment ith ATP, actinomycin D, cycloheximide, methionine ulfoximine, or methionine sulfone did not signifcantly alter the effect of ethionine, but methionine id prevent the enzyme activity increase. These indings indicate that the increase in cystathionine ynthase activity in rat liver caused by ethionine s not a result of *de novo* enzyme synthesis. Ethioine had no effect on enzyme activity in *in vitro* tudies using liver homogenates, suggesting that ne ethionine influence on enzyme activity is not irect. It is possible that some ethionine metabolte causes this effect or that the cystathionine ynthase activity increase, caused by ethionine, was elped by some mediator.

1888 EFFECT OF GASTRO-ENTERO-PANCREATIC ENDOCRINE HORMONES ON THE HISTOGENESIS OF GASTRIC CANCER IN RATS INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE; WITH SPECIAL REFERENCE TO DEVELOPMENT OF SCIRRHOUS GASTRIC CANCER. (Eng.) Tahara, E. (Hiroshima Univ. Sch. Medicine, Kasumi 1-2-3, Hiroshima 734, Japan); Haizuka, S. *Gann* 66(4):421-426; 1975.

Gastrin (50 μg/kg), serotonin (8 mg/kg), histamine (2.5 mg/kg), glucagon (1 mg/kg), and insulin (10 U/kg) were studied for their effects on the histogenesis of gastric cancer in 70 male Wistar rats given N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 80 μg/ml) in drinking water for 40 wk. The five hormones were administered ip at three-day intervals for a total of 20 administrations. Eight of ten rats given gastrin developed adenocarcinomas in the glandular stomach. Six of the eight adenocarcinomas were poorly differentiated, and three of these (observed 40, 46, and 60 wk after the beginning of MNNG administration) were classified as scirrhous carcinoma. Two of the scirrhous carcinomas were located in the fundus and one in the antrum; all exhibited cancerous ulceration. One of the scirrhous carcinomas resembled Borrmann's type IV human gastric cancer in the following ways: (a) Anaplastic carcinoma cells were extensive from the submucosa to the serosa with a remarkable increase of collagen fiber; (b) Diffuse infiltration of tumor was noted in all regions of the glandular stomach; and (c) There was a remarkable embolism of carcinoma cells in the lymphatic cells and in the vein, especially in the fundus. Scirrhous carcinoma was also observed in 2 of 6 animals treated with serotonin. Ulcer formation was more severe in these two cases, and one of the cases (signet-ring cell carcinoma) showed a typical picture of intraperitoneal dissemination. The frequent development of scirrhous carcinoma was not evident in rats given histamine, glucagon, or insulin; and the incidence of gastric cancer in glucagon- and insulin-treated rats was lower than in untreated controls. It is suggested that the development of poorly differentiated adenomas in animals given gastrin may be related to the trophic effect of the hormone, rather than to an increase in acid secretion.

1889 MAMMARY CARCINOGENESIS-ENHANCING EFFECT OF ADRENALECTOMY IN IRRADIATED RATS WITH PITUITARY TUMOR MtT-F4. (Eng.) Clifton, K. H. (Dartmouth-Hitchcock Medical Center, Hanover, N.H. 03755); Sridharan, B. N.; Douple, E. B. *J. Natl. Cancer Inst.* 55(2):485-487; 1975.

The influence of adrenalectomy on mammary oncogenesis in irradiated Fischer (F) rats given grafts of secretory pituitary tumor strain MtT-F4 was investigated. Multiparous female rats (345-390 days old) were divided into six groups. Two groups were unirradiated (1 and 4); two groups (2 and 5) received a total-body dose of 150 rads of "fission neutrons" at a rate of 1.65 rads/min; and the other groups (3 and 6) received a total body dose of 600 rads of gamma-rays from a ^{137}Cs teletherapy source at 45 rads/min. All were inoculated with 0.05 ml of MtT-F4 after radiation. Groups 4, 5 and 6 were adrenalectomized

three days after grafts and given weekly sc injections of 2.5 mg deoxycorticosterone acetate. All animals that died were autopsied, and serum samples were taken for radioimmunoassay of mammotropic hormone (MtH) before the survivors were killed. Groups 1 and 3 had well-developed mammary glands and milk cysts, and no tumors or nodules were observed. Mammary glands of adrenalectomized rats were not secreting milk. Groups 5 and 6 (irradiated and adrenalectomized) had 1.9 tumors per rat (mean); these were carcinomas. Two animals in group 4 had no tumors when adrenal tissue regenerated. Final blood MtH levels were related to final transplantable pituitary tumor size. This is the first report that adrenal insufficiency may promote mammary carcinogenesis. High carcinoma frequencies in adrenalectomized, transplantable pituitary tumor-bearing rats given different radiations strengthens the conclusion that adrenocortical deficiency is important in carcinoma induction.

1890 EFFECTS OF MATERNAL DES INGESTION ON THE FEMALE GENITAL TRACT. (Eng.) Herbst, A. L. (Harvard Medical Sch., Boston, Mass); Scully, R. E.; Robboy, S. J. *Hosp. Pract.* 10(10):51-60; 1975.

More than 250 cases of vaginal or cervical carcinoma among young women were entered in a registry established after the association between these rare cancers and maternal diethylstilbestrol ingestion became apparent. Abnormal vaginal bleeding or discharge was the presenting symptom in most of the cases, but 20% of the patients were asymptomatic, their tumors being detected either by a pelvic examination or by an abnormal cytologic smear. Most clear-cell adenocarcinomas are nodular, polypoid, or papillary. Vaginal adenosis was found near the vaginal clear-cell adenocarcinomas in over 95% of cases; cervical erosion was always present in association with the cervical carcinomas. The type of treatment that is indicated depends on the stage, location, and extent of the tumor. Surgical treatment is preferred to radiation therapy for patients with operable tumors because of their young age and because of the fact that the amount of radiation necessary for cure induces an artificial menopause and may result in vaginal stenosis. Seventeen percent of the cases of Stage I and 51% of the cases of Stage II carcinoma in which lymph nodes were available for examination had metastases. The rates of recurrence and mortality have been higher in these cases than in those with uninvolved nodes. The etiologic factors responsible for the very rare development of clear-cell adenocarcinoma in young women with adenosis are unknown, but some understanding of the histogenesis of these tumors is available. Evidence in favor of a mullerian nature or origin of the clear-cell adenocarcinoma includes (a) the relation of the ovarian tumors to endometriosis and endometrioid carcinomas; (b) the origin of the tumor within the endometrium, where mesonephric remnants have never been identified; and (c) the origin of the diethylstilbestrol-related vaginal tumors superficially (where the mullerian-type epithelium of adenosis is almost always found), rather than deeply (where the bulk of the mesonephric remnants are known to occur). It is suggested that office screening examinations of exposed women begin once they have started to menstruate or by the age of 14 yr. As a result of more widespread screening examinations, more cases are now being discovered in asymptomatic individuals. The fact that all asymptomatic patients are living and well after therapy underscores the importance of screening examinations, even though the likelihood of uncovering a clear-cell adenocarcinoma is very small.

1891 PRIMARY LIVER CANCER ASSOCIATED WITH LONG-TERM OESTROGEN THERAPY. (Eng.) Sotaniemi, E. A. (Dept. Internal Medicine, Univ. Oulu, SF-90220 Oulu 22 Finland); Alavaikko, M. J.; Kaipainen, W. J. *Ann. Clin. Res.* 7(4):287-289; 1975.

A case report is presented for a 60-yr-old woman with primary liver cancer associated with long-term polyestradiol phosphate therapy. Nine years previously the patient had undergone hysterectomy for uterine leiomyomata; since then, estrogen (80 mg) had been administered monthly by im injection. The patient presented with fatigue and a heavy feeling in the right abdomen. Physical examination revealed an enlarged liver with a hard and irregular surface. Liver function tests indicated serum total bilirubin, 1.9 mg/100 ml; SGOT, 225 Wroblewsky U; SGPT, 113 Wroblewsky U; alkaline phosphatase, 10.0 Bessey-Lowry U; sulfobromophthalein retention (45 min), 24.3%; and thrombotest, 64%. Percutaneous liver biopsy revealed an hepatocellular carcinoma. Despite radiotherapy and chemotherapy with 5-fluorouracil, the patient died three months later in hepatic coma. This case suggests a possible association between long-term estrogen therapy for menopausal symptoms and cancer development, and also indicates that estrogen should be taken into account in assessing tumor development in users of oral contraceptives.

1892 EFFECT OF ESTROGEN TREATMENT ON DMBA-INDUCED MAMMARY TUMOR GROWTH AND BIOCHEMISTRY IN INTACT AND DIABETIC RATS. (Eng.) Cohen, N. D. (Univ. Rochester Sch. Medicine and Dentistry, Rochester, N.Y. 14642); Hilf, R.* *Proc. Soc. Exp. Biol. Med.* 148(2): 339-343; 1975.

The effect of estrogen treatment on growth patterns of 7,12-dimethylbenz(*a*)anthracene-induced tumors was examined in diabetic Sprague-Dawley rats. Fifty-day-old rats were intubated with 7,12-dimethylbenz(*a*)-anthracene dissolved in sesame oil for a total of 25 mg in doses of 5 mg/ml once weekly for five weeks; tumors arose 8-15 wk after initial carcinogen administration. Diabetes was induced by a single iv injection of streptozotocin (50 mg/kg). Estradiol valerate was injected sc (1 mg/animal/wk) beginning one day after streptozotocin injection. Tumor regression was defined as a decrease in tumor surface of at least 20%, and tumor growth as an increase of at least 20%. Nucleic acids and enzymes were determined in tumors in intact and diabetic animals. Administration of estra-

iol valerate caused regression of 75% of the tumors tudied in intact animals of all the tumors studied n diabetic rats. The average body weight of the conrol animals, diabetic animals and intact estrogenreated animals were similar. Estrogen treatment of iabetic rats resulted in an increase in DNA levels nd a reduced RNA/DNA ratio in neoplasms when compared o intact control (growing tumor) animals. However, he decrease in RNA caused by the lack of insulin, and he increase in RNA produced by administration of strogen were apparently offset by one another in the umors from diabetic animals treated with estrogen; he resulting level of RNA in these neoplasms was similar to that found in tumors from intact control anials. These data suggest that the mechanism whereby nsulinemia and estrogen therapy cause tumor regresion is different. The activities of pyruvate kinase, hosphofructokinase, glucose-6-phosphate dehydrogense, and 6-phosphogluconate dehydrogenase were reuced in regressing tumors from diabetic animals, hereas no significant effects on enzyme activities ere found after administration of estradiol valerate. umors from diabetic animals treated with estrogen emonstrated activities of pyruvate kinase, phosphoructokinase, and 6-phosphogluconate dehydrogenase hat were significantly reduced below those found in umors of diabetic rats not treated with estrogen. t appears that the effects of estrogen in the diaetic rat enhance some of the responses induced by he diabetic state, perhaps due to a greater sensiivity of tumors to estrogen therapy in the absence f the growth promoting effects of insulin.

893 EFFECTS OF AGE AT CARCINOGEN ADMINISTRATION AND EXPOSURE AS NEONATES TO 17β-ESTRADIOL N SUBSEQUENT GLAND-PAIR DISTRIBUTION OF MURINE MAMARY DYSPLASIAS. (Eng.) Warner, M. R. (Baylor Coll. edicine, Houston, Tex. 77025); Warner, R. L. *J. atl. Cancer Inst.* 54(6):1369-1372; 1975.

he effects of age at carcinogen treatment and expoure to 17β-estradiol on the gland-pair distribution f mammary lesions in BALB/c mice were observed. The ges studied were: 4,5,8, and 10 wk; and 6 and 6½ mo. ach age group was subdivided into groups. One group as given a 40 μg dose of 17β estradiol daily for five ays, beginning 24 hr after birth; others served as arcinogen-injected controls. Dimethylbenz[*a*]anthraene (DMBA) was given to all animals in two 0.5 mg oses at wide intervals. More dysplasias occurred in he secondary gland pair than in third or fourth pair f glands in 19 animals fed DMBA at 4 and 5 wk of age. he incidence of dysplasias between the third and ourth gland pairs did not differ significantly. Esradiol given neonatally along with DMBA at 4 and 5 eeks showed the third and fourth gland pairs had quivalent numbers of dysplasias. Those receiving nly the carcinogen at 8 and 10 wk had equivalent umbers of dysplasias in the third and fourth pairs, ut significantly fewer in the second and third pairs. hen both carcinogen and steroid was given at 8 and 10 k, more dysplasias occurred in the fourth pair. The arcinogen given at 6 and 6½ mo caused the greatest umber of dysplasias in the fourth pair, usually ith equal numbers in the other pairs. In general, usceptibility to dysplasias varied considerably with age. No consistent trend occurred in the distribution of lesions except in the fourth gland pair, regardless of whether or not both steroid and carcinogen were given. However, these findings do not exclude or distinguish between the influence of local or systemic effects of the steroid on the mammary glands.

1894 ARYL AND ANILINE HYDROXYLASES IN RAT NUCLEAR MEMBRANES AFTER PRETREATMENT WITH PREGNENOLONE 16α-CARBONITRILE, PHENOBARBITAL AND METHYLCHOLANTHRENE. (Eng.) Alexandrov, K. (Institut de Recherches Scientifiques sur le Cancer, Boite Postale 8, F-94800 Villejuif, France); Frayssinet, C. *Experientia* 31(7):778-779; 1975.

The induction of aryl and aniline hydroxylases in cell nuclei, nuclear membranes, and microsomes by pregnenolone 16α-carbonitrile, phenobarbital and methylcholanthrene was studied in male WAG rats. One group of rats was given 0.1% sodium phenobarbital in drinking water for two weeks; another group was injected with methylcholanthrene (20 mg/kg in 0.5 ml corn oil) once a day for two days; the third group received a micronized suspension of pregnenolone 16α-carbonitrile (in 2 ml of water), and a trace of Tween 80 (50 mg/kg) at 8-hr intervals (twice daily for two days and once on the third day). The animals were killed 24 hr after the last administration, and the liver was quickly excised and chilled. The liver nuclei were isolated, and the nuclear membranes were prepared by action of heparin on the nuclei. In the microsomes, methylcholanthrene and pregnenolone 16α-carbonitrile induced greater quantities of aryl hydrocarbon hydroxylase than did phenobarbital. In the nuclei and nuclear membranes, neither prenenolone 16α-carbonitrile nor phenobarbital induced the enzyme. Methylcholanthrene, which induced six times more aryl hydrocarbon hydroxylase in the microsomes, induced ten times more enzyme in the nuclei and 15 times more enzyme in the nuclear membranes compared to controls. None of the substances induced aniline hydroxylase in the nuclei or nuclear membranes, although all three were strong inducers of this enzyme in rat liver microsomes. These results support a previous suggestion that the intracellular controls regulating the nuclear membrane enzymes upon action of methylcholanthrene differ from those that control and regulate the microsomal hydroxylase.

1895 GENETIC EXPRESSION OF ARYL HYDROCARBON HYDROXYLASE BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN: EVIDENCE FOR A RECEPTOR MUTATION IN GENETICALLY NON-RESPONSIVE MICE. (Eng.) Poland, A. (Univ. Rochester Sch. Medicine and Dentistry, Rochester, N.Y. 14642); Glover, E. *Mol. Pharmacol.* 11(4):389-398; 1975.

The induction of hepatic aryl hydrocarbon hydroxylase activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was investigated in 14 inbred mouse strains. Female (4- to 9-wk old) mice were given ip injections of *p*-dioxane (0.4 ml/kg) or various doses of TCDD in the same volume of solvent. 3-Methylcholanthrene (80 mg/kg) was administered ip in corn oil

(8 ml/kg). Aryl hydrocarbon hydroxylase activity was assayed in 10,000 x *g* liver supernatants. All strains tested exhibited enzyme activity in response to TCDD. Enzyme activity of genetically 'nonresponsive' strains of mice that fail to respond to 3-methylcholanthrene was induced by TCDD; however, the dose required was greater than for the genetically 'responsive' strains. The dose of TCDD that elicited half the maximal enzyme activity (ED_{50}) in responsive strains, C57BL/6J, BALB/cJ, and A/J, was about 1 nM/kg; in the nonresponsive strains, DBA/2J, AKR/J, and SJL/J, the ED_{50} was at least 10 nM/kg. In the above strains, TCDD at 3 nM/kg failed to induce hepatic aryl hydrocarbon hydroxylase activity in nonresponsive mice, but elicited 70% or more of the observed maximal enzyme activity in responsive strains. In the 14 strains the phenotypic characteristic of aromatic hydrocarbon responsiveness or nonresponsiveness could be detected equally well by a challenge with 3-methylcholanthrene (0.3 mM/kg) or TCDD at 3 nM/kg. In all nonresponsive strains, a 10-fold higher dose of TCDD induced hepatic enzyme activity. The heterozygous offspring of C57BL/6J and DBA/2J parents were distinguishable from both parental strains by an intermediate sensitivity to TCDD. It is concluded that the genetically nonresponsive mice have the structural and regulatory genes necessary for the expression of aryl hydrocarbon hydroxylase. The most likely explanation of the defect in nonresponsive mice appears to be a mutation that results in an induction receptor site with a diminished affinity for inducing drugs, leading to an almost absolute unresponsiveness to 3-methylcholanthrene and a diminished sensitivity to the more potent inducer, TCDD.

1896 INDUCTION OF ARYL HYDROCARBON HYDROXYLASE IN HUMAN FETAL LIVER CELL AND FIBROBLAST CULTURES BY POLYCYCLIC HYDROCARBONS. (Eng.) Pelkonen, O. (Dep. Pharmacol., Univ. Oulu, Finland); Korhonen, P.; Jouppila, P.; Karki, N. *Life Sci.* 16(9): 1403-1410; 1975.

The ability to induce aryl hydrocarbon hydroxylase (AHH) in human fetal liver cells by polycyclic aromatic hydrocarbons was investigated. Fifteen human fetuses were obtained at 9-10 wk gestation through hysterotomy. Hepatic or fibroblast cells were exposed to either benzo(α)pyrene or 3-methylcholanthrene for 24 hr after which AHH activity was determined by a radiometric method. The highest metabolizing capability was with benzo(α)pyrene; the reaction rate was linear up to 15-20 min incubation time and up to 30 μg cellular protein per 0.125 ml of incubation medium. Naphtholflavone at concentrations of 1, 10 and 100 μM inhibited benzo(α)pyrene metabolism from induced cultures, but not control cultures. The AHH induction by benzo(α)anthracene varied from 12.0-88.7 pM/mg cells/10 min and was dose-dependent with maximal induction at levels of 0.5-5.0 mg/100 ml culture medium. The induction varied between hepatic and fibroblast cultures, but there were clear cut differences. These differences may have been due to individual fetal variability. However, it is still suggested that the human fetus may be the most useful carcinogen target, due to tissue accessibility.

1897 THE ROLE OF NADPH-CYTOCHROME *c* REDUCTASE IN MICROSOMAL HYDROXYLATION REACTIONS. (Eng.) Prough, R. A. (Southwestern Medical Sch., 5323 Harry Hines Blvd., Dallas, Tex. 75235); Burke, M. D. *Arch. Biochem. Biophys.* 170(1):160-168; 1975.

An anti-NADPH-cytochrome *c* reductase globulin was purified from sera of rabbits injected with a flavoprotein reductase antigen prepared from Sprague-Dawley rat liver microsomes. The anti-NADPH-cytochrome *c* reductase globulin inhibited cytochrome P-45 and NADPH-dependent hydroxylation of biphenyl by rodent liver and lung microsomal preparations. The inhibition profiles suggested that both the 2- and 4-hydroxylation of biphenyl were mediated by a common NADPH-cytochrome *c* reductase (NADPH-cytochrome P-450 reductase) and that the same flavoprotein species operated in liver and lung microsomes of corn oil- or 3-methylcholanthrene-pretreated Sprague-Dawley rats and Syrian hamsters. An immunologically identical NADPH-cytochrome *c* reductase also apparently functioned in the NADPH-supported metabolism of benzo(*a*)pyrene and ethylmorphine. NADH supported the microsomal metabolism of benzo(*a*)pyrene and ethylmorphine in liver and biphenyl in liver and lung, but the maximal rates of reaction were slower than when supported with NADPH. The K_m of NADH for biphenyl 2- and 4-hydroxylations in control hamster liver microsomes was approximately 5 mM. Anti-NADPH-cytochrome *c* reductase globulin inhibited NADH-supported biphenyl 2- and 4-hydroxylase activities in corn oil- or 3-methylcholanthrene-pretreated rats and hamsters, even at NADH concentrations as low as 2.25 mM. These results indicate that the same flavoprotein reductase species that mediated NADPH-dependent biphenyl hydroxylase donated at least one electron for the NADH-supported hydroxylation.

1898 THE ISOLATION AND CHARACTERIZATION OF POLYCYCLIC HYDROCARBON-BINDING PROTEINS FROM MOUSE LIVER AND SKIN CYTOSOLS. (Eng.) Sarrif, A. M. (Med. Sch., Univ. Wisconsin, Madison); Bertram, J. S.; Kamarck, M.; Heidelberger, C. *Cancer Res.* 35(3):816-824; 1975.

The major protein to which metabolites of methylcholanthrene are covalently bound was purified from C3H mouse liver cytosol. The purification procedure involved successive fractionations on Sephadex G-25, DEAE-cellulose, and Sephadex G-100. Following the last step, the protein was subjected to carboxymethylcellulose chromatography, a second Sephadex G-100 fractionation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The properties of the protein were identical to those of mouse skin *h*-protein, which may be a primary target of carcinogenic hydrocarbon metabolites during transformation to cancer. It had a molecular weight of 44,000, consisted of two subunits of molecular weight 20,000 each, had an isoelectric point (pI) of 8.5-8.6, and a sedimentation coefficient of 3.6 S. These properties are rather similar to those of ligandin, a rat hepatic protein that binds carcinogenic metabolites, steroid anionic metabolites, bilirubin, and exogenous organic anions. However, the *h*-protein and ligandin consistently gave different pI values

verage of 8.3 and 8.9, resp.). The mouse *h*-protein s not similar in physical properties to the rat ver azo dye carcinogen-binding "slow h_2-5S" pro-in. Two minor basic proteins (molecular weight ound 44,000 each), to which methylcholanthrene tabolites are covalently bound, were separated from e *h*-protein by carboxymethyl-cellulose chromato-aphy. Preliminary results indicated that these o minor proteins are identical to ligandin. A otein to which methylcholanthrene is noncovalently und was also identified in the acidic fraction of e mouse liver and skin cytosols. It had a molecu-r weight of 60,000, a pI of 5.0, and a sedimenta-on coefficient of 4.5 S. The protein may be sim-ar to a previously-described protein to which rcinogenic hydrocarbons bind specifically *in vitro*. ether any of these proteins play a role in carcin-enesis is yet to be determined.

99 RELATIONSHIP OF HYPERPLASIA TO CANCER IN 3-METHYLCHOLANTHRENE-INDUCED MAMMARY TU-ROGENESIS. (Eng.) Fisher, E. R. (Shadyside Hosp., ttsburgh, Pa. 15232); Shoemaker, R. H.; Sabnis, A. *b. Invest.* 33(1):33-42; 1975.

e evolution of 3-methylcholanthrene-induced rat mmary tumors was studied using light microscopy. mmary tumors were induced in 40 Wistar-Furth rats the instillation of 20 mg of carcinogen by gas-ic tube twice weekly for six weeks. A seriation changes proceeding from early hyperplasia (8 days) advanced hyperplasia and cancer were indistin-ishable, being characterized by nuclear chromatin umping, prominent nucleoli, cytoplasmic polyribo-mal aggregates and pseudopodal extensions, and ominent Golgi structures. These features, as ll as the lack of limiting lamina basalis, al-wed for their distinction from the cells of early perplasia. Further, only nodules of both advanced perplasia and cancer were transplantable in syn-neic recipients. Chromosomal aberrations were alitatively similar in cells obtained from all sions but were progressively more frequent. Al-ough these findings do not allow for the discrim-ation of advanced hyperplasia and cancer, they dicate their close pathogenetic relationship as ll as the very early occurrence of chromosomal terations in the development of mammary cancer in is model system.

00 HISTONE MODIFICATION IN LIVER AFTER AD-MINISTRATION OF INDUCERS OF MIXED FUNC-ON OXIDASE ACTIVITY. (Eng.) Procaccini, R. L. ledical Coll. Georgia, Augusta, Ga. 30902); Bres-ck, E. *Chem. Biol. Interact.* 11(6):523-533; '75.

cause of the suggestion that histones may func-on in conjunction with other chromosomal pro-ins to control rates of RNA and protein synthe-s, the phosphorylation and acetylation of rat ver histones *in vivo* were determined after admin-tration of phenobarbital or 3-methylcholanthrene. le Sprague-Dawley or Charles River rats received an ip injection of phenobarbital sodium (75 mg/kg) or 3-methylcholanthrene (20 mg/kg); controls received either saline or corn oil, the vehicles for phenobarbital and 3-methylcholanthrene, respectively. No changes were observed in the phosphorylation profile of histones separated by gel electrophoresis after treatment (1, 2, 3, or 6 hr) with either of these two inducing agents. However, after two hours of treatment with either phenobarbital or 3-methylcholanthrene, a significant increase in acetylation of histones was noted, particularly in the $F_2a_1$and (F_3, F_2b, F_2a_2) arginine-rich regions (33 and 38% increase, respectively). It is suggested that this increase that precedes the enhancement in RNA synthesis may play a role in the alteration of transcription in liver.

1901 ABNORMAL SYNTHESIS OF MITOCHONDRIAL DNA IN THE PRESENCE OF *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE *IN VITRO*. (Eng.) Koike, K. (Cancer Inst., Japanese Foundation for Cancer Res., Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan); Kobayashi, M.; Fujisawa, T.; Tanaka, S. *Biochim. Biophys. Acta* 402(3):351-362; 1975.

The *in vitro* effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) on mitochondrial DNA (mtDNA) synthesis was studied using isolated liver mitochondria from newborn Donryu rats. From the kinetics of the incorporation of [^{3}H]thymidine into the acid-insoluble material, MNNG neither stimulated nor inhibited the DNA synthesizing activity of mitochondria. The activity observed in the presence of MNNG was inhibited by *N*-ethylmaleimide (1 mM) and actinomycin D (0.5 μg/ml). By the band velocity sedimentation in CsCl/ethidium bromide, the properties of the nascent mtDNA formed in the presence of MNNG were analyzed. The nascent DNA-containing molecule was not found in the closed-circle fraction, and essentially detected in the open-circle fraction. This change of the template was blocked by *N*-ethylmaleimide but not by actinomycin D, suggesting a conversion of the closed-circular template to the open-circular one by single-strand cleavage(s). From the band sedimentation in alkaline CsCl, the number of nascent higher molecular DNAs was increased, but the molecules were all of relatively lower molecular weight. However, the formation of nascent fragments was inhibited. The alkaline CsCl equilibrium centrifugation analysis revealed that the nascent DNA synthesized in the presence of MNNG consisted of both light and heavy components. The results suggest that MNNG exerts its effect on the mtDNA synthesis by modifying the intrinsic mechanism of discontinuous synthesis, since the conversion of the template DNA molecule from the closed- to open-circular form and the continuous polymerization of the nascent higher-molecular DNA on such a relaxed template were characteristic events *in vitro*.

1902 EFFECTS OF NERVE GROWTH FACTOR ADMINISTRATION ON N-ETHYL-N-NITROSOUREA CARCINO-

GENESIS. (Eng.) Stahn, R. (Sch. Medicine, U.C.L.A., Los Angeles, Calif. 90024); Rose, S.; Sanborn, S.; West, G.; Herschman, H. *Brain Res.* 96(2):287-298; 1975.

The possible existence of a cocarcinogenesis model for tumor induction in the nervous system was investigated by observing whether nerve growth factor in concert with either a general (methylcholanthrene) or a neurotropic (ethylnitrosourea) carcinogen could induce sympathetic neuronal tumors. Pregnant C57BL/6 mice received nerve growth factor (5 µg/g) sc from days 10-20 of pregnancy. Either ethylnitrosourea (5 µg/g intragastrically) or methylcholanthrene (5 µg/g, sc in sesame oil) were given on days 11, 14, and 17 of pregnancy. After birth all animals received nerve growth factor (5 µg/g, sc) for 30 days. A second experiment followed a similar protocol, but the dosages of carcinogens and nerve growth factor were increased to 10 µg/g. Transplacental tumor induction was not attempted in rats because of the large amount of nerve growth factor that would be required. Ethylnitrosourea (30 or 90 µg/g) was administered on the fifth day postnatally by gastric injection to BD-IX rats. Nerve growth factor had no cocarcinogenic effect with either methylcholanthrene or ethylnitrosourea on the sympathetic nervous system of the mouse; this was true when it was administered transplacentally, postnatally, or both transplacentally and postnatally. At a dose of 30 µg/g ethylnitrosourea, nerve growth factor did not shorten the latent period (36 wk for ethylnitrosourea alone, 34 wk for ethylnitrosourea with nerve growth factor) for tumor induction of BD-IX rats. A 25% reduction in latent period (from 31-24 wk) was brought about by nerve growth factor for tumor appearance in the rats receiving 90 µg/g ethylnitrosourea. In both cases the frequency of urogenital tumors was increased as a result of nerve growth factor, at the apparent expense of neural tumors. It is concluded that the existence of a cocarcinogenesis model for tumor induction in the nervous system is not demonstrated by these experiments.

1903 BREAKAGE OF A DNA-PROTEIN COMPLEX INDUCED BY 4-NITROQUINOLINE 1-OXIDE, 4-NITROPYRIDINE 1-OXIDE, AND THEIR DERIVATIVES IN CULTURED MOUSE FIBROBLASTS. (Eng.) Andoh, T. (Inst. Med. Sci., Univ. Tokyo, Japan); Ide, T.; Saito, M.; Kawazoe, Y. *Cancer Res.* 35(3):521-527; 1975.

The effects of a number of 4-nitroquinoline 1-oxide (4NQO) and 4-nitropyridine 1-oxide (4NPO) derivatives of varying carcinogenic potencies on the scission of proteins linking DNA were studied in cultured L x P3 mouse fibroblasts. Cells were treated in monolayer for 30 min at 37 C and cellular DNA was examined by neutral sucrose gradient centrifugation. The more strongly carcinogenic agents (e.g., 4NQO, 2-methyl-4NQO, 6-methyl-4NQO, 6-chloro-4NQO, and 4-hydroxyaminoquinoline 1-oxide) all induced scission at 10^{-5} M, while 5×10^{-5} M was required for the same effect by less strongly carcinogenic agents (3-methyl-4NQO, 6-*n*-butyl-4NQO, 6-*n*-hexyl-4NQO, 6-*tert*-butyl-4NQO, and 6-carboxy-4NQO). Some noncarcinogens (8-nitroquinoline 1-oxide, 4-hydroxyquinoline 1-oxide, 4-aminoquinoline 1-oxide, and 6-nitroquinoline) were unable to induce scission, while others (3-nitroquinoline 1-oxide, 5-nitroquinoline 1-oxide, and 5-nitroquinoline) resulted in scission only at concentrations greater than 10^{-4} M. Cytotoxicity was expressed only at concentrations greater than those at which scission was produced. That some noncarcinogens produced positive results at high concentrations, suggests that they may be weak carcinogens. The results show that carcinogenicity of 4NQO and 4NPO derivatives paralleled quite well the activities of inducing breakage of the DNA-protein complex; this suggests that the latter might be involved in the initiation of carcinogenesis. The authors suggest the system as a method for the detection of environmental carcinogens.

1904 SERYL-tRNA SYNTHETASE AND ACTIVATION OF THE CARCINOGEN 4-NITROQUINOLINE 1-OXIDE. (Eng.) Tada, M. (Aichi Cancer Cent. Res. Inst., Nagoya, Japan); Tada, M. *Nature* 255 (5508):510-512; 1975.

The characteristics of the 4-hydroxyaminoquinoline-1-oxide (4HAQO) activator enzyme were investigated. The enzyme was purified from bakers yeast, and required ATP, L-serine, Mg^{2+} and a sulphydryl protective agent for maximal activity. However, using paper electrophoresis, no significant conversion of L-serine was observed, suggesting the reaction was catalyzed by seryl-transfer RNA synthetase (seryl-tRNA synthetase). The 4HAQO and seryl-tRNA synthetase activities could not be separated by DEAE cellulose, Sephadex G-200, or by sucrose density gradients, as relative reaction rates changed with equal magnitude. Both activities were heat stable. It is concluded that seryl-tRNA synthetase also catalyzes the binding of 4HAQO to nucleic acid. A proposed model suggests that seryl-AMP is made on the seryl-tRNA synthetase molecule, and that 4HAQO reacts with seryl-AMP bound on the enzyme producing seryl-4HAQO. This is supported by the occurrence of binding of 4HAQO to nucleic acid in the absence of the enzyme. The detailed kinetics of this reaction could not be examined, due to rapid hydrolysis. The activated 4HAQO was too unstable to be isolated. It is suggested that 3HAQO may be activated by both seryl- and prolyl-tRNA synthetases in rat tissue. The synthetases may have a unique conformation, enabling them to aminoacylate the N-hydroxy group of the carcinogen; the first activation step may be the formation of N-hydroxy-derivatives, followed by N-hydroxyesterification.

1905 A NEW APPROACH FOR INDUCTION OF PANCREATIC NEOPLASMS. (Eng.) Pour, P. (Univ. of Nebraska Medical Center, Omaha, Nebr.); Kruger, F. W.; Althoff, J.; Cardesa, A.; Mohr, U. *Cancer Res.* 35(8):2259-2268; 1975.

The Syrian golden hamster was used for pancreatic carcinogenesis and tumor histogenesis studies. Eight-week-old hamsters were given weekly sc injections of equitoxic doses (50% lethal dose) of di-*n*-propylnitrosamine, 2-hydroxypropyl-*n*-propylnitros-

amine (2-HPPN), 2-oxopropyl-*n*-propylnitrosamine (2-OPPN), methyl-*n*-propylnitrosamine (MPN), and 2,2'-dihydroxy-di-*n*-propylnitrosamine (DHPN). Animals were sacrificed when moribund, and step or serial pancreatic sections were stained and examined. Although di-*n*-propylnitrosamine produced no carcinogenic effects in the pancreas, its assumed metabolites did. A high incidence of pancreatic tumors occurred with DHPN. All treated animals developed ductal adenomas; 80-100% developed ductal carcinomas; and 17% developed acinar-cell carcinomas. Most pancreatic neoplasms were microscopic and multifocal. Single neoplasms were located in the pars cranialis (8%), pars dextra and corpus (24%), and pars sinistra (8%). The majority (75%) of the larger neoplasms occurred in the pars cranialis. Microscopically, the adenomas were of ductal origin and of wide variety. In contrast to the benign lesions, the intraductal carcinomas were characterized by the proliferation of fairly uniform cells with frequent secondary glandular formations, budding, and focal necrosis. Morphologically, the adenomas included cystic-papillary adenocarcinoma, gelatinous carcinoma, tubular carcinoma, mucinous carcinoma, signet-ring cell carcinoma, and large-cell carcinoma. Some metastasis to regional lymph nodes or to distant organs was also noted. Minimum latent periods ranged from 15-30 wk. The distribution, multiplicity and some biological characteristics of these tumors are comparable to previously reported observations in man; however, by contrast with most cases in humans, the majority of the neoplasms originated in regions other than the head of the pancreas. Thus, the hypothesis that pancreatic neoplasms develop as a result of the reflux of bile into the pancreatic duct is apparently invalid.

1906 NONSPECIFIC INHIBITION OF DNA REPAIR BY PROMOTING AND NONPROMOTING PHORBOL ESTERS.

(Eng.) Langenbach, R. (Univ. Nebraska Medical Center, 42nd and Dewey Ave., Omaha, Nebr. 68105); Kuszynski, C. *J. Natl. Cancer Inst.* 55(4):801-802; 1975.

The effect of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and its nonpromoting structural analogue, 4-*O*-methyl-12-*O*-tetradecanoylphorbol-13-acetate (Me-TPA), on *N*-acetoxy-2-acetylaminofluorene-elicited DNA repair and replicative DNA synthesis was measured in normal human fibroblasts from a 25-yr-old woman, cells passaged 5-10 times. Both esters inhibited DNA repair synthesis. At 1.7 μM, Me-TPA gave 31% inhibition, and TPA, 37%. At 17 μM, TPA gave 50% inhibition of replicative DNA synthesis; Me-TPA gave 43%. Inhibition of DNA repair synthesis may not be a major factor in the mechanisms of action of tumor promoters.

1907 ANGIOSARCOMA OF THE LIVER ASSOCIATED WITH FOWLER'S SOLUTION (POTASSIUM ARSENITE).

(Eng.) Lander, J. J. (Washington Univ. Sch. of Medicine, 660 South Euclid Ave., St. Louis, Mo. 63110); Stanley, R. J.; Sumner, H. W.; Boswell, D. C.; Aach, R. D. *Gastroenterology* 68(6):1582-1586; 1975.

A 43-yr-old Caucasian male with an angiosarcoma of the liver associated with ingestion of 10-15 drops/day of Fowler's solution (potassium arsenite) for 15 yr is described. Upper gastrointestinal hemorrhage, recurrent hemoperitoneum, hepatic failure, and subsequent appearance of an angiosarcoma of the skin characterized the clinical course. Selective angiography demonstrated features consistent with both hepatoma and cavernous hemangioma. Review of the literature shows that basal cell and squamous cell skin cancers are the most common neoplasms associated with arsenic. Cirrhosis after exposure to arsenic has been reported. Fowler's solution was recommended for treatment of psoriasis as recently as 1965, and arsenic has been used in pesticides. Physicians should be aware that arsenic-related angiosarcoma and cirrhosis may occur long after the ingestion of arsenic.

1908 MOUSE DERMAL STUDY OF SMOKE CONDENSATE FROM "CHEMOSOL"-TREATED CIGARETTES.

(Eng.) Gargus, J. L. (Hazleton Lab., Inc., Vienna, Va. 22180); Sullivan, J. B.; Habermann, R. T.; Copeland, J.; Everly, J. *Toxicol. Appl. Pharmacol.* 33(3):568-574; 1975.

The incidence of skin tumors in mice following repeated dermal applications of cigarette smoke condensate from control tobacco and tobacco treated with "chemosol" (1 g citric acid, 10 ml H_2O, 1 ml deuterium oxide) was studied. Eight hundred male and female weanling ICR Swiss mice were treated with benzo(*a*)-pyrene (20 g, positive controls), tobacco condensate No. 27 (controls), tobacco condensate No. 64 ("Chemosol"-treated), acetone (vehicle controls), or nothing (negative controls). A 2 x 3 cm area on the dorsal skin was treated three times weekly for 740 days. Selected tissues were examined microscopically. Both the "Chemosol"-treated and control tobaccos were blends of flue-cured Burely, Oriental, and Maryland tobacco. The nicotine determinations averaged 77.6 mg/g in the "Chemosol"-treated smoke condensate and 76.6 mg/g in the control condensate, and the benzo(*a*)pyrene value was 0.75 ng/mg in the "Chemosol"-treated condensate and 0.76 ng/mg in the control condensate. One hundred and five of the 200 control mice developed a total of 168 skin tumors, with a latent period of 434 days. Eighty-one of these tumors were diagnosed: 54 carcinomas, 23 papillomas, two adenomas, one fibrosarcoma, and one mast cell sarcoma. Ninety-seven of the 100 positive control mice developed tumors with a mean latent period of 162 days; all ten tumors examined were diagnosed as squamous cell carcinomas. No characteristic lesions developed in the negative and vehicle controls. Of the 200 "Chemosol"-treated mice, 106 developed 165 tumors with a mean latent period of 372 days. Of the 88 tumors examined, 58 were carcinomas, 18 were papillomas, one was an adenoma, ten were fibrosarcomas, and one was a hemangiosarcoma. It is concluded that the smoke condensate derived from "chemosol"-treated tobacco is comparable to that derived from untreated tobacco in the induction of tumors in mice following repeated dermal applications.

1909 CHARACTERIZATION OF HUMAN CELLS TRANSFORMED *IN VITRO* BY URETHANE. (Eng.) Benedict, W. F. (Child. Hosp. Los Angeles, Calif.); Jones, P. A.; Laug, W. E.; Igel, H. J.; Freeman, A. E. *Nature* 256(5515):322-324; 1975.

Urethane-treated and untreated cultures derived from two cell lines obtained from siblings with von Recklinghausen's disease were characterized in an attempt to disprove the possibility that a few tumor cells in the original population had been selected for by urethane. One transformed colony (designated "urethane") was derived from a single morphologically altered focus in a control culture treated with 1.1 x 10^{-2}M urethane. A second transformed colony (designated "urethane/FeLV") was derived from a single focus obtained in a urethane-treated control culture preinfected with feline leukemia virus (FeLV). Trypsin-Giemsa banding showed no abnormal metaphases in the untreated control cells, while all metaphases in the urethane cell line at passage 30 were aneuploid. Most metaphase cells contained 94 or 95 chromosomes. An abnormal marker chromosome was found in 19 of 20 metaphases analyzed. An additional marker chromosome was found in 14 of 20 metaphases. Similar marker chromosomes were found at passage 259. The urethane/FeLV line also had an aneuploid chromosomal pattern at passage 25, which was different from that of the urethane cell line because most metaphases contained 68-71 chromosomes and no metaphases had the two marker chromosomes seen in the urethane cell line. Four additional marker chromosomes, however, not found in the urethane cell line were seen at various frequencies. The intracellular and extracellular fibrinolytic activities of the control cells and the two urethane-treated lines were examined. The control cells contained little fibrinolytic activity (that is, the level found in normal human fibroblast cultures), whereas the transformed cells showed marked fibrinolytic activity. In seven animals injected with 2 x 10^6 control cells, no tumors developed in five months. A similar number of cells from the urethane line, however, produced rapidly growing fibrosarcomas within three weeks. It is concluded that chemical transformation of human cells does occur, and is frequently expressed as foci or morphologically altered cells; tissue culture conditions, however, are usually inadequate for the establishment of long term cell lines from these transformed cells.

1910 *IN VIVO* REPAIR OF RAT LIVER DNA DAMAGED BY 3-HYDROXYXANTHINE. (Eng.) Michael, R. O. (Temple Univ. Sch. Med., Philadelphia, Pa.); Parodi, S.; Sarma, D. S. R. *Chem. Biol. Interact.* 10(1):19-25; 1975.

The induction of strand breaks in liver DNA by 3-hydroxyxanthine was studied *in vivo* in male Wistar rats. The animals were killed at various intervals after the i.p. administration of an aqueous solution of 3-hydroxyxanthine (0, 50, 100, or 200 μg/g), and the sedimentation of the liver DNA in neutral and alkaline sucrose gradients was studied. The thermal denaturation of the liver DNA was also measured spectrophotometrically. 3-Hydroxyxanthine treatment resulted in a reduction in the sedimentation of the liver DNA in both gradients, the degree of DNA fragmentation increasing with the dose of carcinogen administered. Slower sedimentation of the liver DNA in the alkaline sucrose gradient was evident within 4 hr following drug treatment at 50 μg/g, the fragmentation being largely repaired within 24 hr of treatment with 100 μg/kg. Fragmentation and repair of the liver DNA was also seen after administration of 100 μg/g of 3-hydroxyxanthine, using neutral sucrose gradients; significant repair of the fragmented DNA was observed within 24 hr. The thermal denaturation of the liver DNA from 3-hydroxyxanthine-treated rats (100 μg/g) did not vary from control values nor did that of calf thymus DNA incubated *in vitro* with this carcinogen. The data indicate that *in vivo* 3-hydroxyxanthine treatment leads to single- and double-strand breaks in the liver DNA. The mechanism by which this drug induced strand breaks in the liver DNA is unknown, but it is possible that this process may play an important role in the initiation of chemical carcinogenesis.

1911 TESTING FOR POSSIBLE EFFECTS OF CEDAR WOOD SHAVINGS AND DIET ON OCCURRENCE OF MAMMARY GLAND TUMORS AND HEPATOMAS IN C3H-A^{vy} AND C3H-A^{vy}fB MICE. (Eng.) Heston, W. E. (Natl. Cancer Inst., Bethesda, Md.). *J. Natl. Cancer Inst.* 54(4):1011-1014; 1975.

The influence of diet and bedding material on the occurrence of tumors and hepatomas in male and female C3H-A^{vy} mice and in male C3H-A^{vy}fB mice was studied. At four weeks of age, littermates were divided equally into two groups: one was fed Charlick's M164 diet and the other was fed Purina chow. Half of new matings of C3H-A^{vy} mice was maintained on 3/4 pine saw dust and 1/4 cedar shavings; the other half was maintained on saw dust alone. Growth curves showed that all groups fed Charlick's diet grew more rapidly during the early part of life. In males fed Charlick's diet, there was a higher incidence of hepatomas, while in females this diet showed a lower age at which mammary tumors developed. Bedding type did not affect growth rate, hepatoma or mammary tumor incidence. The results thus show that cedar shavings are not carcinogenic. The authors maintain that factors which increase growth rate will also increase tumor occurrence.

1912 INDUCTION OF CHROMOSOME CHANGES IN CHINESE HAMSTER CELLS BY EXPOSURE TO ASBESTOS FIBRES. (Eng.) Sincock, A. (Llandough Hosp., Penarth, Glamorgan CF6 1XW, U.K.); Seabright, M. *Nature* 257(5521):56-58; 1975.

1913 DIFFERENTIAL THERMAL ANALYSIS OF CHRYSOTILE ASBESTOS IN PURE TALC AND TALC CONTAINING OTHER MINERALS. (Eng.) Luckewicz, W. (Avon Products, Inc., Suffern, N.Y. 10901). *J. Soc. Cosmet. Chem.* 26(9):431-437; 1975.

914 MORE ABOUT INTRASANGUINEOUS MUTAGENICITY TESTING [abstract]. (Eng.) Ellenberger, J. Zentrallaboratorium fur Mutagenitatsprufung der FG, Freiburg i. Br., West Germany); Mohn, G. *utat. Res.* 29(2):235-236; 1975.

915 ACQUISITION OF TUMOUR-INDUCING ABILITY BY NON-ONCOGENIC AGROBACTERIA AS A ESULT OF PLASMID TRANSFER. (Eng.) Van Larebeke, . (Laboratorium voor Genetica, Rijksuniversiteit ent, B9000 Gent, Belgium); Genetello, C.; Schell, .; Schilperoort, R. A.; Hermans, A. K.; Hernal-steens, J. P.; Van Montagu, M. *Nature* 255(5511): 42-743; 1975.

916 PROPHAGE INDUCTIVE EFFICIENCY OF ALKY-LATING AGENTS AND RADIATIONS. (Eng.) ussain, S. (Wallenberg Lab., Univ. Stockholm, tockholm, Sweden); Ehrenberg, L. *Int. J. Radiat. iol.* 27(4):355-362; 1975.

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* (Phys): 1977, 1985
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* (Immun): 2093, 2121, 2155, 2170
* (Path): 2201, 2203, 2210, 2231, 2245, 2256, 2258, 2259, 2296
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973 FOREIGN-BODY TUMORIGENESIS: *IN VITRO* ISOLATION AND EXPANSION OF PRENEOPLASTIC CLO-
AL CELL POPULATIONS. (Eng.) Buoen, L. C. (Univ. innesota Medical Sch., Minneapolis, Minn. 55455); rand, I.; Brand, K. G. *J. Natl. Cancer Inst.* 55(3): 21-723; 1975.

he intracellular aberrations connected with the preeoplastic stages of cells acquiring neoplastic autoomy were studied in foreign-body reactive tissues *n vitro*. Foreign-body reactions were induced in oisogenic CBA/H and CBA/H-T6 mice by sc implantaion of 15 X 22 X 0.2 mm unplasticized vinyl chlorde vinyl acetate copolymer films. At six mo post mplantation, implants and unopened tissue capsules ere transferred to recipient animals of the T6ifferent partner strain. After another three mo, art of the film/capsule complex was transferred o (C57BL/10ScSn x CBA/H-T6)F_1 mice for tumor deelopment. Capsule-derived and film-attached cells f the other part were separately cultured. Culures consisting initially of euploid cells were ften gradually replaced by different cells with pecific aneuploid karyotypes which were identical ith, or closely related to, those of the corresonding tumors. The cultured cells implanted in ybrid recipients at different passage numbers freuently gave rise to homologous tumors. Hence, it as possible to prepare *in vitro* cells with preixed specific tumor determinants at different stages of preneoplastic maturation. It is concluded hat analytic studies on foreign-body induced preeoplastic cell populations can be expected to comlement findings on chemically or virus-transformed ells.

974 OCCURRENCE OF HEMANGIOSARCOMAS IN BEAGLES WITH INTERNALLY DEPOSITED RADIONUCLIDES.
Eng.) Benjamin, S. A. (Inhalation Toxicology Res. nst., Lovelace Foundation, Albuquerque, N.M. 87115); ahn, F. F.; Chiffelle, T. L.; Boecker, B. B.; Hobbs, . H.; Jones, R. K.; McClellan, R. O.; Snipes, M. B. *ancer Res.* 35(7):1745-1755; 1975.

eagles (12-14 mo old) were exposed to aerosols conaining relatively soluble (chloride) or relatively nsoluble (fused clay) forms of ^{144}Ce and ^{90}Sr. Fify-five dogs were exposed to $^{144}CeCl_3$, and 15 unexosed dogs served as controls. Seventy-two dogs were xposed to $^{90}SrCl_2$, and 25 served as controls. All ogs were bred in a closed colony and had a common ncestry. Dogs exposed to the ^{144}Ce and ^{90}Sr n both the chloride and fused clay forms developed arious neoplasms at or near the sites of radionuclide ocalization. With the soluble $^{144}CeCl_3$, significant adiation doses were delivered to the lungs, liver, nd skeleton, but after $^{90}SrCl_3$ exposure, the radiaion was delivered predominantly to the skeleton. In ogs exposed to ^{144}Ce and ^{90}Sr in fused clay particles, adiation doses were delivered mostly to the lungs and racheobronchial lymph nodes. The deaths of dogs dyng within two years after exposure were attributable o nonneoplastic radiation-induced lesions in the taret organ systems. At later times after exposure, eoplasms were the major cause of death, again occurring mostly in target organs or adjacent tissues. The incidence of hemangiosarcomas was over 40% in all studies. A pulmonary hemangiosarcoma had developed in every dog that died with a neoplasm before 3.6 yr after exposure to the relatively insoluble radionuclide forms. The authors suggest the possibility that a specific genetic susceptibility to hemangiosarcomas exists, or that the existence of an oncogenic virus (when triggered by radiation) leads to induction of tumors of endothelial origin.

1975 RADIATION DOSE FROM PLUTONIUM DEPOSITED IN MARROW AND BONE OF NORMAL AND CHIMAERIC MICE. (Eng.) Humphreys, E. R. (Medical Res. Council, Radiobiology Unit, Harwell, Didcot, Oxon OX11 ORD, U.K.); Green, D. *Int. J. Radiat. Biol.* 27(6): 561-567; 1975.

The difference in sensitivity in mice to induction of different types of skeletal tumors by plutonium was investigated to determine whether it results from differences in distribution of plutonium in the tissues. ^{239}Pu citrate was injected ip into CBA mice and into CBA mice which had been made chimeric by replacing their hematopoietic bone-marrow with that from another genetically-identical but cytologically-distinct strain. The mice were killed at three intervals up to 90 days after injection, and the deposited ^{239}Pu was determined radiochemically in bone-marrow and in bone. The average radiation dose (integrated over 90 days) was found to be greater in the bones but lower in the marrow of chimeric mice than in the corresponding tissues of the normal CBA mice. It is concluded that the differences in the metabolic behavior of ^{239}Pu and the possible long-term effects which they bring about are induced by the procedures involved in producing chimeras, and that the differences found in the doses received by marrow and by bone fit, qualitatively, previous data on induced malignancy.

1976 DISTRIBUTION OF THE FEMORAL COLONY-FORMING CELLS IN ^{226}Ra-INJECTED MICE. (Eng.) Svoboda, V. (Inst. Hyg. Epidemiol., Prague, Czechoslovakia). *Radiol. Clin. Biol.* 44(2):103-111, 1975.

The question of whether or not the response of colony-C forming cells to the radiation from deposited ^{226}Ra is dependent on marrow localization in bone structures was investigated. Female random-bred ICR mice were injected ip with ^{226}Ra activity levels of 0.02 μCi and/or 0.09 μCi; each of these levels was given in three injections within one week. Peripheral blood counts were made at intervals of 1-2, 4-6, 10-11, 16-18, and 42-43 wk after ^{226}Ra administration. Three separate suspensions of marrow cells were made, including the proximal ends, diaphyses, and the distal ends of femora. Aliquots of 0.2 ml marrow cell suspension were injected into heavily irradiated mice. ^{59}Fe (1 μCi) was later injected into the grafted mice, and its activity was measured in the spleens of irradiated mice. RBC and WBC were decreased in the group treated with 0.02 μCi ^{226}Ra/g, and were extremely depleted in the group treated with 0.09 μCi ^{226}Ra/g. In the later group, 12% of marrow cells were in the proximal ends, 79% were in the shaft, and 9% were in the distal ends of the femora at the second week after nuclide injection.

The cells of active hemopoietic marrow could not be found in the spongious parts of femoral cavities four weeks after injection. ^{59}Fe uptake in recipient spleens was depressed. Colony forming units (CFU) were calculated in the hemopoietic system of the irradiated host, and an extreme and fatal depletion of femoral hemopoiesis was observed. In mice with higher activity, 3.7% of the femoral CFU were in the distal ends, and 10% were in the proximal ends of the bones two weeks after injection. The hemopoietic system is seriously damaged by radiation of ^{226}Ra; the effect is more serious in the spongious structures and greatest in the distal metaphyses and epiphyses.

1977 LUNG CANCER INDUCED IN HAMSTERS BY LOW DOSES OF ALPHA RADIATION FROM POLONIUM-210. (Eng.) Little, J. B. (Harvard Univ. Sch. Public Health, Boston, Mass.); Kennedy, A. R.; McGandy, R. B. *Science* 188(4189):737-738; 1975.

The carcinogenicity to the lung of low levels of ^{210}Po alpha radiation was examined in Syrian golden hamsters, which are resistant to chronic pulmonary infections and have a zero incidence of spontaneous lung tumors. In one series of experiments, the hamsters received 15 weekly intratracheal injections of ^{210}Po adsorbed on 3 mg of ferric oxide carrier particles and suspended in saline; the particles contained 0.25, 1.25, or 5.0 nanocuries (nCi) ^{210}Po yielding, respectively, lifetime exposures of 15, 75, and 300 rads to the lungs. In other experiments, ^{210}Po was administered in saline alone in doses of 1.25 nCi (15 instillations) or 100 nCi (seven instillations), which yielded lifetime exposures of 55 and 1,500 rads, respectively. ^{210}Po given on carrier particles induced lung tumors (epidermoid carcinomas) in 11% of 83 animals receiving 15 rads, in 12% of 82 animals receiving 75 rads, and in 53% receiving 300 rads. Borderline malignancies were noted in 13-62% of the hamsters. After administration on carrier particles, ^{210}Po was retained in the lungs in a distinctly heterogenous pattern, whereas the radioactivity was uniformly distributed throughout the lung parenchyma following administration in saline alone. However, this uniform distribution was not associated with a marked difference in the carcinogenic effect of ^{210}Po. Lung tumors developed in 9% of 101 hamsters receiving 55 rads and in 58% of 38 receiving 1,500 rads from ^{210}Po in saline. Borderline malignancies were present in 17% and 61% of these animals. Cigarette smokers have previously been estimated to receive 20 rads to areas of the bronchial epithelium from deposited ^{210}Po. This study supports the hypothesis that alpha radiation resulting from the ^{210}Po or lead-210 present in cigarette smoke may be a significant causative factor in human lung cancer.

1978 STUDIES ON THE MODIFYING EFFECT OF ULTRAVIOLET RADIATION ON CHEMICAL SKIN CARCINOGENESIS. (Eng.) Stenbäck, F. (Univ. Nebraska Med. Cent., Omaha). *J. Invest. Dermatol.* 64(4):253-257; 1975.

The effect of UV light on chemically induced skin carcinogenesis in female Swiss mice from the Eppley colony was investigated. A series of experiments were performed in which varying doses of 7,12-dimethylbenz(a)anthracene (DMBA) and 3,4-benzo(a)pyrene [B(a)P] were topically applied to the back skin of the mice and in which the sequence and interval between UV treatment and carcinogen application were varied. Repeated treatment with 12 μg DMBA twice weekly for eight wk induced a large number of tumors; DMBA alone induced papillomas and squamous cell carcinomas. With UV irradiation only (5.5×10^7 ergs/cm^2, three hr twice weekly for eight wk), papillomas, squamous cell carcinomas and fibrosarcomas were produced. UV treatment 24 hr before DMBA applications increased the total tumor yield to 37 from 29; after DMBA applications, UV caused a decrease in the tumor yield from 28 to 10, with only papillomas and a fibrosarcoma remaining. Neither a single exposure to UV irradiation (5.5×10^7 ergs/cm^2) nor croton oil (0.02 cc in 2.5% solution, twice weekly for 30 wk) treatment induced tumors; but UV irradiation prior to a single application of B(a)P (100 μg) as an initiating agent, followed by repeated applications of croton oil, increased the neoplastic response. Histologically, the tumors induced by B(a)P and croton oil were benign fibropapillomas and acanthopapillomas, with fibrous stalks covered by proliferating squamous epithelium. The stromal response was more prominent after irradiation. The enhancing effect of a low, nonulcerating dose of UV light (2.8×10^6 ergs/cm^2) on initiation by DMBA (50 μg) in two-stage skin carcinogenesis was also significant. The results do not support the hypothesis that the UV light effect on the hair cycle is significant, as the time period between irradiation and initiation is too short for significant changes in the hair cycle to occur. However, the number of regressing tumors was significant. The increased tumor population possibly represents a lower level of neoplastic transformation induced by the addition of UV light or depending on a specific effect on the host or on its immunologic defense mechanisms.

1979 RECOVERY RATE FOR TUMOR INDUCTION IN RAT SKIN WITH SPLIT DOSES OF ELECTRONS [abstract]. (Eng.) Vanderlaan, M. (New York Univ. Med. Cent., N.Y.); Burns, F. J.; Albert, R. E. *Radiat. Res.* 62(3):598; 1975.

1980 THE DOSE RESPONSE CURVE FOR TUMOR INDUCTION WITH SINGLE AND SPLIT DOSES OF 10 MEV PROTONS [abstract]. (Eng.) Burns, F. J. (New York Univ. Med. Cent., N.Y.); Albert, R. E.; Vanderlaan, M.; Strickland, P. *Radiat. Res.* 62(3): 598-599; 1975.

1981 LATE EFFECTS OF NEUTRON OR GAMMA RADIATION [abstract]. (Eng.) Ainsworth, E. J. (Div. Biol. Med. Res., Argonne Natl. Lab., Ill.); Fry, R. J. M. *Radiat. Res.* 62(3):555; 1975.

1982 MAMMARY NEOPLASIA IN THE RAT AFTER EXPOSURE TO HIGH ENERGY NEUTRONS [abstract]. (Eng.) Montour, J. L. (Med. Coll. Virginia, Richmond). *Radiat. Res.* 62(3):596; 1975.

983 EFFECTS OF GRAFTS OF SINGLE ANTERIOR PITUITARY GLANDS ON THE INCIDENCE AND YPE OF MAMMARY NEOPLASMS IN NEUTRON OR GAMMA RRADIATED FISCHER RATS [abstract]. (Eng.) Clifon, K. H. (Univ. Wisconsin Med. Sch., Madison); ridharan, B. N.; Douple, E. B. *Radiat. Res.* 2(3):596; 1975.

984 EFFECTS OF LOW LEVELS OF RADIATION ON RODENTS AND POTENTIAL EFFECTS IN MAN. Eng.) Warren, S. (New England Deaconess Hosp., oston, Mass.). *Health Phys.* 29(2):251-255; 1975.

985 FLUORESCENT RESPONSE OF CELLS TREATED WITH OLIVOMYCIN AND IRRADIATED WITH 425 M LIGHT [abstract]. (Eng.) Burns, V. W. (Univ. alifornia, Davis). *Radiat. Res.* 62(3):534; 1975.

986 THE EFFECT OF IRRADIATION ON THE RATIO OF CYCLING (P) TO NON-CYCLING (Q) CELLS N EMT6 TUMORS AS DETERMINED BY COLONY LABELING abstract]. (Eng.) Kallman, R. F. (Stanford Univ. ch. Med., Calif.). *Radiat. Res.* 62(3):528; 1975.

1987 POLYADENYLIC ACID-CONTAINING CYTOPLASMIC RNA INCREASES IN X-IRRADIATED NEUROBLASTOMA CELLS IN CULTURE [abstract]. (Eng.) Prasad, K. N. (Univ. Colorado Med. Cent., Denver); Bondy, S. C.; Purdy, J. L. *Radiat. Res.* 62(3): 585; 1975.

1988 EFFECT OF RADIATION ON GENETIC MATERIAL. (Spa.) Elejalde, B. R. (Laboratorio de Genetica, Departamento de Patologia, Universidad Javeriana, Antioquia, Colombia); Restrepo, J. G.; Molina, J. *Antioquia Med.* 25(1):27-35; 1975.

See also:

* (Rev): 1803, 1805, 1834
* (Chem): 1889, 1916, 1963
* (Immun): 2088, 2147, 2148, 2192
* (Epid): 2315
* (Path): 2230, 2287

1989 COMPARISON OF VIRAL RNA SEQUENCES IN ADENOVIRUS 2-TRANSFORMED AND LYTICALLY INFECTED CELLS. (Eng.) Flint, S. J. (Center for Cancer Res., Massachusetts Inst. of Technology, 77 Massachusetts Ave., Cambridge, Mass. 02139); Gallimore, P. H.; Sharp, P. A. *J. Mol. Biol.* 96(1):47-68; 1975.

The complementary strands of fragments of ^{32}P-labeled adenovirus 2 DNA generated by cleavage with restriction endonucleases *Eco*RI or *Hpa*I were separated by electrophoresis. Saturation hybridization reactions were performed between these fragment strands, and unlabeled RNA was extracted from the cytoplasm of adenovirus 2-transformed rat embryo cells or from human cells early after adenovirus 2 infection. The fraction of each fragment strand complementary to RNA from these sources was measured by chromatography on hydroxylapatite. Maps of the viral DNA sequences complementary to messenger RNA (mRNA) in different lines of transformed cells and early during lytic infection of human cells were constructed. Five lines of adenovirus 2-transformed cells were examined. All contained the same RNA sequences, complementary to about 10% of the light strand of *Eco*RI fragment A. DNA sequences coding for this RNA were more precisely located using *Hpa*I fragments E and C and mapped at the left-hand end of the genome. Thus, any viral function expressed in all adenovirus 2-transformed cells, (e.g., tumor antigen) must be coded by this region of the viral genome. Two rat embryo fibroblast lines, F17 and F13, expressed only these sequences; rat embryo fibroblast line 8617 and rat embryo muscle cell line REM also contained mRNA complementary to about 7% of the heavy strand of the right-hand end of adenovirus 2 DNA; the fifth line, T2C4, contained these and many additional viral RNA sequences in its cytoplasm. The viral RNA sequences found in all lines of transformed cells are also present in the cytoplasm of human cells during the early phase of a lytic adenovirus infection. The additional cytoplasmic sequences in the 8617 and REM cell lines also respond to "early" RNA sequences.

1990 ANALYSIS OF EARLY ADENOVIRUS 2 RNA USING ECO R·RI VIRAL DNA FRAGMENTS. *(Eng.)* Craig, E. A. (Washington Univ. Sch. Med., St. Louis, Mo.); Zimmer, S.; Raskas, H. J. *J. Virol.* 15(5):1202-1213; 1975.

Adenovirus 2 RNA synthesized early in the productive *in vitro* infection of KB cell suspensions was analyzed by RNA-DNA hybridization using adenovirus 2 DNA and the six adenovirus 2 DNA fragments generated by digestion with the restriction endonuclease ECO R·R1. Duplex formation between RNA and ^{32}P-labeled viral DNA was assayed by S_1 nuclease digestion. Cytoplasmic RNA annealed 12% of the total viral DNA, 6% of the R1-A fragment, 24% of the R1-B fragment, none of the R1-F fragment, 40% of the R1-D fragment, 13% of the R1-E fragment, and 22% of the R1-C fragment. The early cytoplasmic RNA was composed of two sequence classes: the first was present in greatly reduced quantities 18 hr after infection, and the second was maintained in high concentrations at 18 hr. Hybridization-inhibition experiments indicated that the early cytoplasmic transcripts of R1-D belonged to class II, whereas the R1-C transcripts were class I sequences; the R1-A and R1-B fragments were class I and II sequences. The class I sequences included a 19S RNA transcribed from R1-B and the class II sequences included a 20S RNA derived from R1-D. Nuclear RNA purified from cultures early in infection was annealed with ^{32}P-labeled R1 fragments; with all six fragments the nuclear RNA annealed as much or more of the DNA than did the cytoplasmic RNA. Eco R1-F annealed at least 25% with early nuclear RNA, but no sequences homologous to R1-F were detected in the early cytoplasmic RNA. When the KB cultures were labeled 2-6 hr after infection, at least 5% of the ^3H-labeled early nuclear viral RNA annealed to Eco R1-F. Some of the nuclear transcripts from R1-F appeared to be covalently linked to sequences transcribed from a contiguous region of the genome (Eco R1-B); 8.4% of the RNA selected by hybridization of R1-F reannealed to R1-B, while no more than 1.5% reannealed to R1 fragments A, D, E, or C.

1991 ISOLATION OF DNA POLYMERASE FROM AN ADENOVIRUS 2 DNA REPLICATION COMPLEX. (Eng.) Ito, K. (St. Louis Univ. Sch. Med., Mo.); Arens, M.; Green, M. *J. Virol.* 15(6):1507-1510, 1975.

The purification and some properties of the major DNA polymerase present in the nuclear membrane complex of KB cells were fractionated into cytoplasm, nucleoplasm, and the nuclear membrane complex. The DNA polymerase activity was solubilized by treatment of the complex with Triton X-100 and 0.5 M KCl and centrifuged at 100,000 *g* for one hour. The supernatant fluid was dialyzed against 4 l of 20 mM potassium phosphate buffer, 20% glycerol, 0.1 mM EDTA and 3 mM DTT overnight, and then adsorbed to phosphocellulose. The enzyme was eluted with a linear gradient of 0.02-0.6 M potassium phosphate. The major peak eluted at about 0.18 M potassium phosphate and constituted 85% of the total DNA polymerase activity eluted from the column. The minor peak, which eluted at about 0.11 M potassium phosphate, was variable in amount and may have been due to cytoplasmic contamination. Both the major and minor peak activities utilized poly(A) x $(dT)_{12-18}$ with about five times the efficiency of activated calf thymus DNA. The major phosphocellulose peak enzyme was further purified on DEAE cellulose, where it eluted as a single peak of activity at 0.1 M KCl. The enzyme sedimented as a single symmetrical peak in a glycerol gradient with both calf thymus and synthetic polymer activities. The enzyme had a sedimentation coefficient of about 6.7S, equivalent to a molecular weight of about 125,000. In several different preparations, the glycerol-purified enzyme was purified about 90-fold from the Triton extract, corresponding to approximately a 900-fold purification from the initial cell pellet. The glycerol gradient-purified DNA polymerase of the adenovirus 2 DNA replication complex had greater than 2-fold higher activity with poly(A) x $(dT)_{10}$ as template-primer in the presence of Mn^{2+} than with activated calf thymus DNA in the presence of Mg^{2+}; this distinguishes it from DNA polymerase α and β present in the cytoplasm and

n the nucleus of KB cells. The complete recovery f activity by the Triton-high salt extraction, the pparent lack of DNA-dependant polymerase activity hroughout the purification procedure, and the consistently high ratio of activity with poly(A) x oligo-dT) *versus* calf thymus DNA strongly indicate that the nly DNA polymerases present are γ DNA polymerases.

992 ENHANCEMENT OF ADENOVIRUS INFECTION IN WI-38 AND AGMK CELLS BY PRETREATMENT OF ELLS WITH 5-IODODEOXYURIDINE. (Eng.) Staal, S. P. Natl. Inst. Allergy Infect. Dis., Bethesda, Md.); owe, W. P. *Virology* 64(2):513-519; 1975.

he effect of 5-iododeoxyuridine (IdU) on the replication of adenoviruses in human fibroblasts and African Green Monkey Kidney (AGMK) cells was investigated. oth adenovirus types 7 and 2 recovered from tonsil ulture produced more extensive and rapidly appearing cytopathologic effects (CPE) in WI-38 cells when ells were pretreated with IdU. The increase in titer in adenovirus 7 cultures was 8- to 130-fold, with aximum enhancement at 50 μg/ml IdU after 48 hr. AGMK ells were also sensitive to IdU (20 μg/ml, 48 hr) ielding a 2.4- to 17-fold increase in growth. Pretreatment of WI-38 and AGMK cells increased the number of T-antigen-positive cells by the same order of agnitude as the increased virus yield, indicating he IdU-sensitive restriction occurs prior to T-antigen synthesis and is due to the number of productively nfected cells, rather than increased efficiency of iral synthesis. IdU treatment had no effect on virus bsorption. AGMK cells with IdU enhancement was ound to be additive to Simian virus 40 enhancement f adenovirus yield. Cotreatment of WI-38 cells with dU (50 μg/ml) and cytosine arabinoside (5 μg/ml) for 8 hr before infection caused no enhancing effect; his indicates that IdU incorporation into cellular NA is required for enhancement. Enhancement is postlated to be due to an increased number of cells peritting early virus functions to occur and permitting ore cells to be susceptible to viral transformation. he IdU system may represent a widely distributed cell efense mechanism.

993 HEAT-STABLE VARIANT OF HUMAN ADENOVIRUS TYPE 5: CHARACTERIZATION AND USE IN HREE-FACTOR CROSSES. *(Eng.)* Young, C. S. H. (Inst. irol., Glasgow, Scotland); Williams, J. F. *J. irol.* 15(5):1168-1175; 1975.

he isolation and partial characterization of a eat-stable (*hs*) variant of human adenovirus type (Ad5) is reported. The *hs* mutant was isolated rom the temperature-sensitive mutant (*ts*1), the eat stability of which is similar to that of the ild type. Mutant *ts*1 was inactivated at 52 C and portion of the surviving fraction was seeded on eLa cells to obtain a high-titer stock. A portion f this stock was then inactivated at 52 C and a econd high-titer stock was obtained. Plaques solated 5, 10, and 15 min after heat inactivation of his second stock were tested for heat stability t 52 C. A putative heat-stable mutant was thus btained. The variant was genetically stable, both through vegetative viral passage and through recombination into other genetic backgrounds; this suggests that it arose from a single mutation. Three-factor crosses using this mutant in conjunction with *ts* mutants further suggest that the *hs* mutation lies near the left hand end of the genetic map. The mutant was used to demonstrate the production of reciprocal recombinants in two-factor crosses. The mutational lesion is unknown, but phenotypic mixing occurs in *hs* x hs^+ infections, which suggests that it lies in a gene specifying a virion structural protein. The following phenotypic characteristics were identical in the *hs* and wild-type hs^+ viruses: ability to transform rat embryo cells; ability to induce interferon on chick embryo fibroblasts; frequency of ts^+ recombinants in crosses of the general types *tsx* x *tsy*, *tsx-hs* x *tsy*, *tsx* x *tsy-hs*, and *tsx-hs* x *tys-hs*; inactivation by neutralizing antiserum to Ad5; stability on storage at -20 and -70 C; ability to form infection centers, particle to PFU ratios; and yields of virus at 32, 37, and 38 C. The *hs* marker can be used in three-factor crosses to aid in the production of an unambiguous genetic map of Ad5.

1994 THE INITIAL NUCLEOTIDE SEQUENCE OF DNA TRANSCRIBED FROM AVIAN MYELOBLASTOSIS VIRUS 70 S RNA BY RNA-DEPENDENT DNA POLYMERASE. (Eng.) Eiden, J. J. (Duke Univ. Med. Cent., Durham, N. C.); Bolognesi, D. P.; Langlois, A. J.; Nichols, J. L. *Virology* 65(1):163-172; 1975.

The function of the major 4S RNA in RNA tumor viruses and the initial nucleotide sequence of DNA transcribed from avian myeloblastosis virus and Rous sarcoma virus 70S RNA were determined. Denaturation of avian myeloblastosis virus 70S RNA resulted in the release of one major and several minor species of 4S RNA, which were separated by polyacrylamide gel electrophoresis. Quantitative analysis showed that 1-2 copies of the major 4S RNA species were associated with each 70S RNA complex. The T_1 ribonuclease fingerprint of the major 4S RNA species had a complexity characteristic of a molecule approximately 80 nucleotides long, indicating that the major 4S RNA fraction from the polyacrylamide gels is comprised of a single transfer RNA species. Quantitative data indicate that only the major 4S RNA species was present in the 70S RNA complex in an amount sufficient to satisfy the requirements for a single unique primer for DNA synthesis *in vitro*. Direct evidence for the priming activity of the major 4S RNA species was obtained by carrying out a reaction in which reverse transcriptase was used to label nonradioactive 70S DNA in the presence of only one deoxynucleoside triphosphate, [α-^{32}P]-deoxyadenosine triphosphate. The isolated 70S RNA was heat-dissociated and subjected to two-dimensional polyacrylamide gel electrophoresis. There was only one major component present, and it had the electrophoretic mobility expected for 4S RNA. The covalent linkage between primer RNAs and nascent DNAs involved an adenosine residue at the 3'-termini of the RNAs and a deoxyadenosine residue at the 5'-termini of the DNAs. By nearest neighbor analysis, a single unique nucleotide sequence, dApdApdTpdGpdApdApdGpdC_{OH} was determined to be present at the 5'-termini of the

nascent DNA transcripts. It is concluded that the primer activity for DNA polymerase in the 70S RNA complex of avian myeloblastosis virus resides in a single unique species of 4S RNA, which is distinct from the corresponding Rous sarcoma virus primer.

1995 BINDING PROPERTIES OF AVIAN MYELOBLASTOSIS VIRUS DNA POLYMERASES TO NUCLEIC ACID AFFINITY COLUMNS. (Eng.) Grandgenett, D. P. (St. Louis Univ. Sch. Med., Mo.); Rho, H. M. *J. Virol.* 15(3);526-533; 1975.

The binding properties of the α and αβ DNA polymerases of avian myeloblastosis virus (AMV) was studied by means of various nucleic acid affinity chromatography columns. The functional roles of the α and β subunits were also studied by comparisons of the effectiveness of various natural and synthetic templates. A method for the analysis and purification of the RNA-directed DNA polymerase of AMV was developed, using an immobilized oligo(dT) moiety annealed with poly(A). The α and αβ DNA polymerases of AMV bound effectively to poly(A) x oligo(dT)-cellulose. αβ DNA polymerase bound to both poly(A)- and poly(C)-cellulose columns, whereas α DNA polymerase did not. Neither bound to oligo(dT)-cellulose. αβ DNA polymerase bound tighter to poly(A) x oligo(dT)-cellulose and to DNA cellulose columns than did α DNA polymerase. The αβ DNA polymerase reversibly transcribed AMV 70S RNA approximately five times faster than did the DNA polymerase in the presence of Mg^{2+}, and was equally efficient in the presence of Mn^{2+}. α DNA polymerase transcribed 9S messenger RNA somewhat better than did αβ with either metal ion. It is suggested that the αβ polymerase, rather than the α DNA polymerase, functions *in vivo*.

1996 AMINO ACID SEQUENCE HOMOLOGY OF MAMMALIAN TYPE C RNA VIRUS MAJOR INTERNAL PROTEINS. (Eng.) Oroszlan, S. (Flow Laboratories, Inc., Rockville Md. 20852); Copeland, T.; Summers, M. R.; Smythers, G.; Gilden, R. V. *J. Biol. Chem.* 250(16): 6232-6239; 1975.

The NH_2-terminal amino acid sequence of the major group-specific antigen, the major internal virion protein (p30; approximate molecular wt 30,000) of several mammalian type C RNA viruses was determined by the Edman degradation procedure using an automated protein sequenator. All of the proteins analyzed showed a high degree of over-all sequence homology and also contained specific regions or single residues. All p30s began with the sequence prolyleucylarginyl (Pro-Leu-Arg) and had an invariant, conserved region from residues 11 to 24. In this region, only a single amino acid difference appeared between the cat and mouse p30s. At position 17, alanine was found in the cat, and serine in all the mouse proteins. This homologous region started at position 10 for RD-114 and baboon virus p30s, and at position 18 in the protein of the virus isolated from gibbon ape. The region extending from residue 4 to 10 showed considerable variability between p30s isolated from different mammalian species. Of 24 residues compared, only a single amino acid difference was found between six different mouse p30s. At position 4, three had leucine, two had alanine, and one had serine. The comparative sequence data demonstrate that the viral p30s are products of related genes in the viruses from various mammalian species.

1997 NUCLEIC ACID HOMOLOGY OF MURINE XENOTROPIC TYPE C VIRUSES. (Eng.) Callahan, R. (Natl. Cancer Inst., Bethesda, Md.); Lieber, M. M.; Todaro, G. J. *J. Virol.* 15(6):1378-1384; 1975.

Nuclec acid hybridization was used to compare the homology of xenotropic virus genomes recovered from various mouse strains. A single-stranded [^{3}H]DNA transcript of BALB/c xenotropic viral RNA was annealed to cytoplasmic RNA from cells producing other xenotropic viruses; the final extents of hybridization (determined with S_1 nuclease) were used as a measure of the degree of relatedness of the hybridized nucleic acid sequences. Two major subclasses of xenotropic murine type C viruses were distinguished. The most frequently encountered subclass (murine leukemia virus-X^α) included isolates from BALB/c, C57BL/6J, C58/J, AKR/J, CBA/J, and DBA/2J inbred strains, and from the Asian feral mouse subspecies *Mus musculus molossinus*. The other subclass (murine leukemia virus-X^β) consisted of viruses isolated from the NIH Swiss and NZB/BINJ strains. Thus, significant polymorphism exists among the endogenous type C virogenes of a single species, *Mus musculus*. Murine leukemia virus-X^α genes were found in strains that also have endogenous mouse-tropic viruses (either N-tropic, B-tropic, or both), whereas the murine leukemia virus-X^β subclass was restricted to mouse strains from which mouse-tropic viruses have not yet been isolated. This suggests that mouse-tropic virogenes may have been derived from xenotropic murine leukemia virus-virogenes. This suggestion is consistant with a model that proposes that all murine endogenous type C virogenes developed from a common ancestor through gene duplication and subsequent mutational events. A common ancestor accounts for the partial homology of all murine type C viral genomes. The branch leading to the murine leukemia virus-M virogenes diverged from the murine leukemia virus-X^α stem sometime after the divergence of the murine leukemia virus-X^α and murine leukemia virus-X^β virogenes.

1998 TRANSPLANTABLE MURINE TUMORS RELEASE MOUSE-TROPIC AND XENOTROPIC TYPE-C VIRUSES. (Eng.) Lieber, M. M. (Natl. Cancer Inst., Bethesda, Md.); Sherr, C. J.; Todaro, G. J. *Int. J. Cancer* 15(4):555-560; 1975.

A study was conducted to determine whether transplanted murine tumors produce type-C viruses. Excised tumor tissue from mice bearing transplantable tumors were co-cultivated with indicator host cell lines NIH/3T3, BALB/373, and rabbit corneal cell line SIRC in medium containing 2 μg/ml polybrene. The cell lines were tested for type-C virus by an *in vitro* assay for supernatant reverse transcriptase activity. Reverse transcriptase activity was confirmed as originating from murine type-C virus

by using IgG, developed against partially purified Rauscher murine leukemia virus reverse transcriptase, to inhibit supernatant reverse transcriptase activity from the test cultures. The activity was considered of type-C virus origin if inhibition was over 90%. Eleven of 20 transplantations were found to produce type-C virus giving a positive transcriptase activity. Only two tumors, the C3HBA mammary adenocarcinoma (passaged in C3H/HeJ mice) and the C1300 neuroblastoma of A/J mice did not release detectable type-C virus. These results did not indicate whether the tumor released a mixture of viruses of standard host range types or viruses of combined tropism, and did not estimate the amounts of virus product. Transplanted murine tumors can thus transmit infectious type-C virus which may generate a wide range of epiphenomena unrelated to presense of the tumor *per se*. The authors suggest caution in handling transplantable tumors releasing such viruses because of their possible transmission to other species, including man.

1999 HETEROGENEITY OF SURFACE ANTIGENS OF ENDOGENOUS TYPE C VIRUS-PRODUCING CELL SUBLINES DERIVED FROM A CLONAL LINE OF BALB/3T3 CELLS. (Eng.) Aoki, T. (Natl. Cancer Inst., Bethesda, Md. 20014); Herberman, R. B.; Liu, M. *Intervirology* 5(1/2):31-42; 1975.

Virus-associated cell surface antigens PC1, X.1, GCSA, and MEV-SA1 and viral envelope antigens xVEA, xl-VEA, and sub-gsVEA were used as markers of virus populations present in various sublines of the BALB/c embryonic fibroblast BALB/3T3 clone A31 line. Of four spontaneously transformed sublines, two released N-tropic endogenous type C viruses spontaneously after long-term culture, and each had distinct antigenic patterns. Treatment with 5-bromodeoxyuridine (BrdU, 100 μg/ml, 24 hr) resulted in the detection of X-tropic viruses in all four sublines. The expression of these X-tropic viruses was associated with two different antigenic patterns. When the clonal rabbit corneal SIRC cell line was infected with the X-tropic viruses obtained after BrdU treatment, the cells acquired detectable amounts of only one of the antigenic markers. The use of viral envelope antigens and cell surface antigens as virus markers demonstrates the complexity and heterogeneity of cell surface antigens associated with endogenous viruses, even in sublines derived from the same cloned line.

2000 DNA OF EPSTEIN-BARR VIRUS. I. COMPARATIVE STUDIES OF THE DNA OF EPSTEIN-BARR VIRUS FROM HR-1 AND B95-8 CELLS: SIZE, STRUCTURE, AND RELATEDNESS. (Eng.) Pritchett, R. F. (Dep. Med., Univ. Chicago, Ill.); Hayward, S. D.; Kieff, E. D. *J. Virol.* 15(3): 556-569; 1975.

The size, structure and relatedness of Epstein-Barr virus (EBV) DNA purified from the extracellular virus produced by B95-8 and HR-1 cells were studied. These are lymphoblastoid cell lines, the B95-8 derived after exposure of marmoset WBC to extracts of a cell line established from a patient with transfusion-acquired infectious mononucleosis, and the HR-1 derived from a biopsy specimen of a patient with Burkitt's lymphoma. Velocity sedimentation analysis, length measurements carried out under the electron microscope, isopycnic banding, DNA-DNA reassociation kinetics analysis and thermal chromatography were carried out. The relative sedimentation velocity in neutral sucrose gradients of native EBV DNA was 101 x 10^6. A value of 105 x 10^5 for the estimated molecular weight of native EBV DNA was obtained by measurement of the length of the molecule relative to form II PM2 DNA. Less than half of EBV DNA sedimented as a single band in alkaline sucrose gradients in the region expected for DNA of 50 x 10^6 daltons following alkali denaturation. Both intact EBV HR-1 and EBV-B95-8 DNA banded at 1.718 g/cm^3, corresponding to 58% guanine plus cytosine. EBV-B95-8 DNA yielded two bands following shearing: a larger band at 1.716-1.717 g/cm^3, and a band (about 25% of the DNA) at 1.720 g/cm^3. HR-1 DNA had more than 97% of the B95-8 DNA sequences. Thermal chromatography on hydroxyapatite showed that hybrid DNA molecules formed between ^{3}H-labeled virus HR-1 DNA and EBV-HR-1 DNA or EBV-B95-8 DNA had identical thermal stability. The B95-8 DNA lacked 12-15% of the HR-1 DNA sequences. It is suggested that genetic complexicity may be associated with enhanced transformation potential.

2001 EPSTEIN-BARR VIRUS GENOMES WITH PROPERTIES OF CIRCULAR DNA MOLECULES IN CARRIER CELLS. (Eng.) Adams, A. (Karolinska Inst., Stockholm, Sweden); Lindahl T. *Proc. Natl. Acad. Sci. U.S.A.* 72(4):1477-1481; 1975.

The DNA conformation of Epstein-Barr Virus (EBV) genomes in Raji cells is reported. Raji cells were grown in suspension cultures in RPMI 1640 medium with 10% fetal bovine serum. DNA was prepared by cell lysing using 3% Sarkosyl and 1% pronase, and was precipitated with CsCl. Raji [^{3}H]DNA in CsCl was supplemented with 10 ng/ml of sheared *Klebsiella pneumoniae* [^{14}C]DNA as reference marker and centrifuged for 65 hr at 33,000 rpm, 20 C. Fractions were collected and removed for determinations of ^{3}H and ^{14}C radioactivity, and for the ability to hybridize with [^{32}P]RNA complementary to EBV DNA. Fractions were further analyzed on neutral glycerol gradients (with phage T_4 [^{14}C]DNA added as reference), and on CsCl/propidium diiodide gradients. EBV DNA sedimented more slowly than T_4 DNA at 0.94-0.95 times the rate of phage DNA. Approximately 80% of the total EBV DNA applied to the CsCl gradient was recovered in the high density region with a peak at 1.716 g/cm^3. Free viral DNA sedimented at 1.718 g/cm^3. Using neutral glycerol gradients the majority of EBV DNA sedimented into two distinct peaks, 65S and 100S. When Raji [^{3}H]DNA from the 95-110S regions were analyzed, 8-10% of the total DNA banded as a small peak at the position of the covalently closed circular DNA. The remaining DNA banded as linear or nicked circular DNA. The small peak contained 50% of the total EBV DNA recovered. Using the CsCl/propidium gradient, 95% of the EBV DNA sequences present banded together with the cellular DNA. It appears that latent EBV DNA has the properties of a mammalian episome. Both nonintegrated and integrated viral DNA sequences can be isolated from carrier cells.

2002 CYTOTOXIC EFFECTOR CELLS SPECIFIC FOR B CELL LINES TRANSFORMED BY EPSTEIN-BARR VIRUS ARE PRESENT IN PATIENTS WITH INFECTIOUS MONONUCLEOSIS. (Eng.) Svedmyr, E. (Karolinska Institutet, S-104 01 Stockholm 60, Sweden); Jondal, M. *Proc. Natl. Acad. Sci. USA* 72(4):1622-1626; 1975.

The capacity of lymphocytes isolated from 12 patients with infectious mononucleosis (IM) to kill a variety of cell lines that do not carry the Epstein-Barr virus (EBV) genome was studied. Lymphocytes from three EBV-seropositive individuals and the IM-infected individuals were isolated on Ficoll-Isopaque gradients. One-fourth of the lymphocytes were kept on RPMI 1640 medium plus 10% heat-activated fetal calf serum without further purification, and the remainder were treated by rosette sedimentation to remove the C'-receptor cells. The chromium-51 release cytotoxicity test was performed with the medium supplemented with 5 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes) buffer. All lymphocytes of IM patients were cytotoxic to KAPLAN, whether complement (C') receptor-positive lymphocytes were removed or not. EBV-genome-positive lines were sensitive to IM lymphocytes, and this effect did not disappear after removal of the non-specific cytotoxic cells. There was a clear difference in cytotoxic activity between the lymphocytes of different IM patients, but most of the cytotoxic effects disappeared after fractionation. Normal lymphocytes stimulated *in vitro* with autologous EBV genome-positive lymphoblastoid cell lines showed extensive blastoid formation. Such cells were ten times more efficient killers for the EBV genome-positive and -negative lines than were fresh IM lymphocytes. This cytotoxic action did not disappear upon removal of C' receptor-bearing cells, neither against EBV genome-positive or -negative lines. These results indicate the presence of effector cells in the peripheral blood of patients with acute IM. Such effectors may play a role in clinical manifestations, as well as in the self-limitation of IM.

2003 IMMUNOFLUORESCENCE AND ANTI-COMPLEMENT IMMUNOFLUORESCENCE ABSORPTION TESTS FOR QUANTITATION OF EPSTEIN-BARR VIRUS-ASSOCIATED ANTIGENS. (Eng.) Reedman, B. M. (Queensland Inst. Med. Res., Herston, Australia); Hilgers, J.; Hilgers, F.; Klein, G. *Int. J. Cancer* 15(4):566-571; 1975.

The estimation of virus capsid antigen (VCA), early antigen (EA), and Epstein-Barr virus (EBV)-determined nuclear antigen (EBNA) in extracts of lymphoblastoid cells using immunofluorescence absorption techniques is described. Cell lines QIMR-WIL, Raji, P3HR1, Daudi, Molt-4, and leukemic cells were used as target cells for the titration of VCA in cell extracts. Cytosine arabinoside (5 μg/ml) was included in the medium to prevent DNA synthesis when EA levels were examined. Smear slides with 8-10 separate wells were prepared by coating standard microscope slides with a hydrophobic teflon film. For VCA and EA tests, [illegible] were allowed to dry overnight; cells [illegible] quickly in high concentra- [illegible] cells/ml). Dilutions of F-Simiya or F-Nathan were used in direct immunofluoresence for EA- or VCA-positive cells. Target cells were stained with fluorescein-conjugated anti-human complement ($antiB_{1c}/B_{1a}$) and counterstained for the anti-complement immunofluorescence method. The endpoint for EBNA was read as the final dilution of serum giving distinct nuclear fluorescence compared with control-containing reference serum. All the EBV-carrying lymphoblastoid cell lines, irrespective of producer status, contained EBNA. Absorption tests indicated the VCA, EA, and EBNA from various sources were antigenically closely related. Absorbing activity was not found in cells without the EBV genome. It was not valid to compare relative levels of VCA, EA and EBNA in individual cell extracts because different reference sera, target cells, and techniques were involved. Immunofluorescence absorption is dependent upon the affinity of binding and the actual amounts of antigens. The techniques are useful for following individual antigens during purification, and for demonstrating antigenic indentity in cell extracts.

2004 NO EVIDENCE FOR PARTICLES ENCAPSULATING RNA-INSTRUCTED DNA POLYMERASE AND HIGH MOLECULAR WEIGHT VIRUS-RELATED RNA IN HERPESVIRUS INDUCED TUMOURS OF NON-HUMAN PRIMATES. *(Eng.)* Laufs, R. (Hygiene-Institut der Universitat D-34 Gottingen, Kreuzbergring 57 West Germany); Steinke, H. *J. Gen. Virol.* 27(2):239-245, 1975.

Twelve tumorous spleens or lymph nodes from nonhuman primates with malignant lymphoma induced by oncogenic herpesvirus (*Herpesvirus saimiri* and *H. ateles*) were tested for the presence of particles encapsulating RNA-instructed DNA polymerase. These spleens and lymph nodes were negative for ^3H-DNA-RNA complex in the 70S region of a glycerol sedimentation velocity gradient. This complex was present in mouse tumor induced with Rauscher leukemia virus (RLV) and mouse mammary tumor virus (MMTV). The presence of inhibition in the monkey tumor directed against DNA polymerase was excluded by reconstruction experiments using NC-37 cells infected with simian carcinoma virus type 1 (SSV-1). Only ^3H-DNA was obtained from NC-37 cells infected with SSV-1 precipitated with ethanol and analyzed on Cs_2SO_4 gradients. Annealing experiments showed that 40% of the input ^3H-DNA from SSV-1 hybridized to polysomal RNA from NC-37 cells infected with SSV-1 but no ^3H-DNA hybridized to the polysomal RNA from herpesvirus-induced tumors. Attempts to demonstrate partial expression of the oncornavirus genome and determine an interspecies antigen related to monkey oncornaviruses were also negative. There was thus no evidence for the presence of RNA tumor virus in herpesvirus-induced malignant lymphoma of nonhuman primates.

2005 REPLICATION OF *HERPESVIRUS SAIMIRI* IN CULTURED LYMPHOCYTES OF INFECTED OWL MONKEYS (*AOTUS TRIVIRGATUS*): AN ELECTRON MICROSCOPIC AND IMMUNOFLUORESCENT STUDY. (Eng.) Giddens, W. E., Jr. (Reg. Primate Res. Cent., Seattle, Wash.). *Lab. Invest.* 32(4):492-502; 1975.

Viral replication was studied in short-term lymphocyte cultures from three control and five Herpesvirus

saimiri (HVS)-infected male and female owl monkeys. The lymphocytes were separated on Ficoll-Hypaque gradients, incubated in suspension cultures, and prepared for electron microscopic and immunofluorescent study 24, 48, or 72 hr after the beginning of culture (AC). Lymphocytes from control and infected monkeys were also cocultivated with Vero cells and analyzed for evidence of a cytopathic effect, and the buffy coat from control and infected whole blood was studied by electron microscopy. Within 50 days after the lymphocytes were cultured, 4 of the 5 HVS-infected monkeys had died of malignant lymphoma and lymphocytic leukemia. HVS virions were demonstrated by electron microscopy, and HVS antigens were demonstrated by immunofluorescence in 2 of 5 cultures 24 hr AC, in 4 of 5 cultures at 48 hr AC, and in all five cultures at 72 hr AC. There was good agreement between the electron microscopic and immunofluorescent data. None of the control cultures and none of the buffy coat preparations contained HVS virions or antigens. Ultrastructually, most of the virus particles were nucleocapsids within the nuclei of the lymphocytes; enveloped virions were seldom observed. There was some evidence that a higher percentage of HVS-containing lymphocytes was correlated with a poorer prognosis for the monkey. HVS was isolated from all five infected monkeys after cocultivation with Vero cells; the control cocultivations were negative. It is likely that a much larger percentage of circulating lymphocytes was latently infected with HVS than these data indicate.

2006 SPONTANEOUS LYMPHOMA ASSOCIATED WITH *HERPESVIRUS SAIMIRI* IN OWL MONKEYS. (Eng.) Rabin, H. (Litton Bionetics, Inc., Kensington, Md.); Neubauer, R. H.; Pearson, G. R.; Cicmanec, J. L.; Wallen, W. C.; Loeb, W. F.; Valerio, M. G. *J. Natl. Cancer Inst.* 54(2):499-502; 1975.

Lymphoma (two cases) and lymphoproliferative disease (one case) were reported in a group of seven Peruvian owl monkeys (*Aotus trivirgatus*) imported into a colony and quarantined. The lymphoma occurred in monkeys caged together. The animals died at 59 days (At 723-I with lymphoma), 144 days (At 724-I with lymphoproliferative disease) and 171 days (At 718-I with lymphoma) after arrival. Virus isolation from tumor cells resulted from cocultivation of primary cultures of lymphoid tumor cells from monkey At 718-I with VERO cells. Virus isolation from monkey At 724-I kidney cells was made from trypsinized kidney tissue that spontaneously developed plaques suggestive of herpes simplex virus (HSV)-induced cytopathology. Virus identification was made by indirect immunofluorescence and serum neutralization with owl and squirrel monkey anti-HSV and human anti-Epstein-Barr virus (EBV) sera. Terminal sera from monkey At 718-I, from monkey 724-I, and from the four monkeys without clinical evidence of disease were tested for antibodies to HSV and EBV. The samples were taken from 175-206 days after arrival. Antibody to HSV was found in sera from the two diseased monkeys positive for HSV but not in sera from the four clinically normal monkeys. Antibody to EBV was also found in the serum from the monkey with lymphoma. These data support previous findings, and indicate that spontaneous lymphomas, apparently associated with *Herpesvirus saimiri* infection, can occur in owl monkeys.

2007 VARIATION IN SUSCEPTIBILITY OF DIFFERENT CELL TYPES TO TEMPERATURE-SENSITIVE HOST RANGE MUTANTS OF HERPES SIMPLEX VIRUS TYPE 2. (Eng.) Koment, R. W. (Milton S. Hershey Med. Cent. Pennsylvania State Univ., Hershey); Rapp, F. *Virology* 64 (1):164-169; 1975.

The ability to produce cytopathologic effects and to replicate at 39 C in various cell types were examined for four conditional lethal mutants (69, 74, 46 and 41) of herpes simplex virus type 2 (HSV-2). Primary cell cultures were prepared from epithelioid cells (hamster, mouse and rabbit kidneys) and from fibroblastoid cells (hamster, mouse, rabbit and human lung tissues) and inoculated with virus at an infective multiplicity of 2. All cell types underwent transformation by HSV-1 and all four mutants replicated in all cell types at 33 C, producing total cytopathic effect. HSV-1 induced complete cytopathic effect in mouse embryo fibroblast cultures and replicated at 39 C. Although all mutants replicated in these cells at 39 C, no discernable cytopathic effect was observed. All epithelioid cells and human and rabbit fibroblastoid cells were permissive to virus replication at 39 C. The results indicate that these herpes simplex virus type 1 mutants are temperature-sensitive and have host-range properties.

2008 QUANTITATIVE ASSAY FOR TRANSFORMATION OF 3T3 CELLS BY HERPES SIMPLEX VIRUS TYPE 2. (Eng.) Duff, R. (Milton S. Hershey Med. Cent., Pennsylvania State Univ., Hershey); Rapp. F. *J. Virol.* 15(3):490-496; 1975.

A quantitative assay was developed to evaluate the transforming potential of the herpes simplex virus type 2 (HSV-2), using virus inactivated by UV irradiation, on Swiss/3T3 host cells. Swiss/3T3 cells were tested for their ability to support the normal replicative cycle of either HSV-2 or the type 1 virus (HSV-1), and neither virus replicated. However, extensive virus-specific cytopathic effects developed from 6-12 hr after infection, followed by death of the host cell. Swiss/3T3 cells were infected in suspension by UV-irradiated HSV-2. The cells were trypsinized five days after infection. HSV-2 inactivated with UV irradiation (2, 4, 6, and 8 min) induced cell death in the absence of replication, but no cell death occurred after infection by virus irradiated for 10, 12, or 14 min. Cells infected with UV-inactivated virus (10 and 12 min) continued to replicate past the contact-inhibited monolayer. Transformed cells with an epithelioid or fibroblastoid morphology were identified after treatment with Wright stain, and isolated. The maximum frequency of HSV-2-induced transformation was 3 x 10^6 plaque-forming U/transformed focus. Type C virus particles were seen only after ten cell culture passages of the HSV-2-transformed cell lines. It is concluded

that this system is useful for determining the events regulating transformation by the herpes simplex virus, as well as the mechanism of virus latency within the transformed cell.

2009 DEOXYRIBONUCLEOTIDE METABOLISM IN HERPES SIMPLEX VIRUS INFECTED HeLa CELLS. (Eng.) Cheng, Y.-C. (Yale Univ. Sch. Medicine, New Haven, Conn. 06510); Goz, B.; Prusoff, W. H. *Biochim. Biophys. Acta* 390(3):253-263; 1975;

The effect of Rolly No. 11 strain herpes simplex virus infection (10-20 plaque-forming U/cell) of HeLa cells in culture on deoxynucleotide metabolism and the level of various enzymes concerned with the biosynthesis of DNA was investigated. Of 18 enzyme activities studied, thymidine kinase, DNA polymerase, and deoxyribonuclease were markedly augmented, a finding in agreement with previous reports. Deoxycytidine kinase, ribonucleotide reductase, thymidylate kinase, and deoxycytidylate deaminase activities, in contrast with previous reports did not increase; the actiivties of the other enzymes studied, also did did not increase. While most of the radioactivity derived from [^{14}C]thymidine in the acid-soluble fraction of the uninfected cells was present as deoxythymidine triphosphate, that present in the infected cells was primarily in the form of deoxythymidine monophosphate. Thus, in the infected cell, deoxythymidylate kinase is a rate-limiting enzyme in the biosynthesis of deoxythymidine triphosphate. A marked increase in the pools of the four naturally occurring deoxynucleoside triphosphates (deoxythymidine triphosphate, deoxycytidine triphosphate, deoxyadenosine triphosphate, deoxyguanosine triphosphate) was found. The rate of formation of the virus-induced enzymes was determined, as were the various nucleoside triphosphate pools, and the other phosphorylated derivatives of thymidine; a maximum was reached for all these components between 6 and 8 hr postinfection. Although an apparent greater synthesis of DNA occurred in the uninfected cells, when the specific activity of the radioactive deoxythymidine triphosphate was taken into account, there was actually a greater rate of DNA synthesis in the infected cells, with the peak at 8 hr postinfection.

2010 STRUCTURE AND FUNCTION OF HERPESVIRUS GENOMES. I. COMPARISON OF FIVE HSV-1 AND TWO HSV-2 STRAINS BY CLEAVAGE OF THEIR DNA WITH *ECO R* I RESTRICTION ENDONUCLEASE. (Eng.) Skare, J. (Yale Univ. Sch. Med., New Haven, Conn.); Summers, W. P.; Summers, W. C. *J. Virol.* 15(4):726-732; 1975.

Various cleavage patterns obtained with several different strains of Herpes simplex virus (HSV), types 1 and 2, using the restriction nuclease *Eco* RI, were compared. African green monkey kidney cells were infected with HSV-1 strains KOS, 14-012, MP, F, and CI 101, and HSV-2 strains 333 and 186. Viral DNA was labeled with 1 mCi $P^{32}O_4$. Endonuclease *Eco* RI cleaved each DNA tested into a relatively small number of fragments. The molecular weights of the fragments were measured by quantitative electron microscopy using the ϕX174 RF as a length standard (3.7 x 10^6 daltons). Migration *via* electrophoresis in 0.5% agarose gels showed the migration distance to be inversely proportional to the logarithm of the molecular wt of the fragment for fragment masses less than 13 Mdaltons. The fraction of label in fragments of HSV-1 strain F was determined to estimate its fraction of the total genome; the results showed that the fragments were not all equimolar. All of the HSV-1 viruses, except 14-012 and CI 101, were different in the 3-4 Mdalton size range. Among the more subtle differences were those in size and molarity of fragments 9.5 and 9.6 Mdaltons, plus differences in the minor bands near 8 and 11 Mdaltons. The HSV-2 patterns showed similarities only in three fragments; these were near 10, 3.1, and 0.4 Mdalton. It appeared that the strains of HSV-1 differed from the strains of HSV-2 at most of their cleavage sites, and that a 15% mismatch in base pairing occurred in the homologous regions. Although the origin of the minor restriction fragments is not clear, it is suggested that genetic duplication could result in fragment sites of unequal recombination, producing DNA molecules with large segments in common, but with other heterogeneous regions. The availability of well-defined fragments of HSV DNA can permit more detailed examination.

2011 REGRESSION OF FELINE SARCOMA VIRUS-INDUCED SARCOMAS IN DOGS. I. MORPHOLOGIC INVESTIGATIONS. (Eng.) Slauson, D. O. (Sch. Vet. Med., Univ. California, Davis); Osburn, B. I.; Shifrine, M.; Dungworth, D. L. *J. Natl. Cancer Inst.* 54(2): 361-370; 1975.

Sequential morphologic changes in developing and regressing feline sarcoma virus (FeSV)-induced tumors were studied in mongrel and beagle dogs. Eight weaned beagles received either 2.0 or 3.0 gEq FeSV sc (0.5 ml vol/mEq); 12 newborn beagles received 1.0 gEq FeSV sc (0.5 ml vol/mEq); and seven newborn mongrels received 2.0 gEq FeSV sc (1.0 ml vol/mEq). There were three uninoculated littermate controls. Tumor growth was monitored daily, and tissue samples from biopsy and necropsy were histologically examined. Tumors arose only at the site of inoculation and developed in two (25%) of the weaned beagles; in ten (83.3%) of neonatal beagles and in one (14.3%) mongrel. The average time between inoculation and first palpable tumor growth was 13.3 days. Large, rapidly growing fibrosarcomas developed in 62% of the dogs, 31% had smaller tumors that regressed more rapidly, and one dog developed a medium-sized tumor. The mean life spans for the smaller and larger tumors were 22 and 63 days, repectively. No metastases were detected. The tumor life span was divided into approximately equal periods of growth and regression. In the first phase, a latent period, thymic atrophy and lymph node necrosis were seen. The proliferation I phase showed a loosely cellular myxomatous tumor, and a marked rise in the mitotic index. The tumor in the proliferation II phase was densely cellular and fibromatous with collagenous stroma, and there was a further rise in the mitotic index. Focal necrosis and polymorphonuclear infiltration characterized the tumor in the regression I phase. Also observed were lymphocytic cuffs and lymph node hyperplasia. The mitotic index continued to rise, but more slowly. The regression II phase was charac-

terized by a mixed infiltrate, fibrosis, lymph node hyperplasia, and a drop in the mitotic index. In the recovery phase there was tissue scarring and fibrosis. Regression histopathology was not uniform, and it is suggested that regression was effected by different immunologic mediation systems.

2012 MAREK'S DISEASE HERPESVIRUSES. I. PRODUCTION AND PRELIMINARY CHARACTERIZATION OF MAREK'S DISEASE HERPESVIRUS A ANTIGEN. (Eng.) Long, P. A. (Dept. Microbiology and Public Health, Michigan State Univ., East Lansing, Mich. 48824); Kaveh-Yamini, P.; Velicer, L. F. *J. Virol.* 15(5):1182-1191; 1975.

A method was developed for the large-scale production of Marek's disease herpesvirus (MDHV) A antigen in quantities sufficient to permit its purification and characterization. Roller bottle cultures of primary duck embryo fibroblasts (DEF) were seeded with MDHV strain GA-infected DEF. The roller bottles were then washed and reincubated in serum-free medium, which was harvested daily for maximum yield of MDHV-A antigen. The antigen was analyzed by immunodiffusion and sedimentation of sucrose gradients and was assayed for pathogenicity and virulence in 1-day-old RPL chicks. Radioactively labeled antigen was further studied by immune co-precipitation analysis and autoradiography. The antigen was stable at pH between 2 and 11.5 and appeared to be a glycoprotein based on its sensitivity to trypsin; the specific immune co-precipitation of labeled amino acids and glycosamine, and the detection of radioactive glycosamine by immunodiffusion and autoradiography. The apparent molecular weight of the glycoprotein antigen was estimated to be 44,800 by gel filtration on Sephadex G-200 in the presence of 2 M urea and 0.05% Brij 35. The antigen aggregated and was reduced in titer during storage, but dissociated readily and regained titer in 1 or 2 M urea and 0.05% Brij 35. Fresh, unaggregated antigen and dissociated stored antigen sedimented at 3.5S on sucrose gradients. It is unlikely that MDHV-A antigen is interferon or an interferon-like substance.

2013 MAREK'S DISEASE HERPESVIRUSES. II. PURIFICATION AND FURTHER CHARACTERIZATION OF MAREK'S DISEASE HERPESVIRUS A ANTIGEN. *(Eng.)* Long, P. A. (Dep. Microbiol. Public Health, Michigan State Univ., East Lansing); Clark, J. L.; Velicer, L. F. *J. Virol.* 15(5):1192-1201; 1975.

A method is described for the purification of Marek's disease herpesvirus A (MDHV-A) antigen and several of the antigen's physical and chemical properties are reported. The antigen was purified more than 200-fold with a 24% recovery by ion exchange chromatography, isoelectric focusing, and preparative polyacrylamide gel electrophoresis. It was further analyzed by electrophoresis on analytical polyacrylamide disc gels and immunodiffusion. The antigen had an isoelectric point of 6.68 in the presence of 1 M urea and 0.05% Brij 35, a nonionic detergent, and approximately 6.5 in the absence of dissociating agents. The purified antigen migrated as a single broad band on analytical polyacrylamide gels; this band stained for both protein and carbohydrates. Both immunodiffusion analysis and polyacrylamide gel electrophoresis indicated that the antigen had not been purified to homogeneity, the former technique indicating that the purified antigen was still contaminated with at least 2 other antigens. Antibody to MDHV-A antigen was prepared in a rabbit. Adsorption of the rabbit serum with sonically treated extracts of uninfected cells removed antibody to only one contaminant, while further adsorption with concentrated culture medium from uninfected cells and/or calf serum removed antibody to the remaining contaminant. Immunodiffusion analysis of the resulting antiserum yielded a single line which was identical with a line formed by chicken serum. The data indicate that the MDHV-A antigen is a glycoprotein.

2014 INHIBITION OF BOVINE LEUKEMIA VIRUS RELEASE. (Eng.) Onuma, M. (Dept. of Veterinary Science, Univ. of Wisconsin, Madison, Wis. 53706); Olson, C.; Baumgartener, L. E. *J. Natl. Cancer Inst.* 54(5): 1199-1202; 1975.

The inhibited production of virus in bovine leukemia virus(BLV)-infected cell cultures containing sera from cattle infected with BLV or with the adult form of lymphosarcoma was studied. Sera were obtained from cattle naturally infected with BLV, from normal cattle, and from inoculated cattle. The supernatant media of a cultured BLV-infected fetal lamb spleen cell line (FLSv+) were concentrated and examined for antigenic activity by single radial immunodiffusion. An antigen was also prepared from the cells. All tests were done with the same BLV-infected cell line, and each test serum was examined in conjunction with 20% fetal calf serum (FCS). The virus release inhibition (VRI) test indicated that sera from three cows with the adult form of lymphosarcoma and 5 of 7 cattle inoculated with BLV inhibited virus release from a cell line of fetal lamb spleen infected with BLV. The sera from seven cattle with calf and thymic forms of lymphosarcoma, and six control cattle failed to repress virus release or inhibit virus replication; this was indicated by precipitin ring comparison. In addition, FLSv+ cells grown with a serum-inhibiting virus recovered their ability to release virus when subsequently given media containing FCS. Cell-surface antigen of FLSv+ cells was detected by a direct immunofluorescence test. The authors suggest that antibody of the adult form of lymphosarcoma reacts to cell-surface antigen, and that this reaction is probably detected by immunofluorescence and VRI tests.

2015 REGRESSION OF FELINE SARCOMA VIRUS-INDUCED SARCOMAS IN DOGS. II. IMMUNOLOGIC INVESTIGATIONS. (Eng.) Slauson, D. O. (Sch. Vet. Med., Univ. California, Davis); Osburn, B. I.; Shifrine, M.; Dungworth, D. L. *J. Natl. Cancer Inst.* 54(2): 371-377; 1975.

Tumor-specific, cell-mediated and humoral reactions were sequentially monitored *in vitro* for beagle and mongrel dogs with feline sarcoma virus (FeSV)-induced sarcomas. The results were compared with histopathologic markers of regression (cytotoxic

assays) detected *in vivo*. The sequential development of serum blocking activity and virus-neutralizing antibody activity against FeSV were also followed. All immunologic parameters were detectable in tumor-bearing dogs. *In vitro* cell-mediated immunity remained high throughout the time tumors were relatively free of lymphoid infiltrates. Lymphoid cells did not infiltrate regressive tumors in any appreciable numbers until the later phase of regression. The ability of lymphoid cells to cause *in vitro* tumor cell destruction did not correlate with the biological behavior of the tumors. The level of cytotoxicity *in vitro* was equally high during progressive tumor growth and tumor regression. Serum blocking activity paralleled the rise in lymphocytotoxicity; however, at a somewhat lower level it rose during the later stages of regression and after regression. Blocking activity was also present at equal or slightly higher levels during lymphoid infiltration of tumors. The sequential development of cytotoxic antibody activity compared to tumor growth showed a peak of serum activity just before a recognizable serum cytotoxic antibody. Histology during the first phase of regression showed focal necrosis with neutrophilic infiltration. Neutralizing antibody activity showed a parallel development with cytotoxic antibody activity. It is suggested that antigen-antibody reactions involving neutrophil-mediated regression were important in bringing about tumor cell destruction.

2016 DIFFERENTIAL HOST RANGE OF VIRUSES OF FELINE LEUKEMIA-SARCOMA COMPLEX. (Eng.) Sarma, P. S. (Natl. Cancer Inst., Bethesda, Md.); Log, T.; Jain, D.; Hill, P. R.; Huebner, R. J. *Virology* 64(2):438-446; 1975.

The susceptibility or partial resistance of heterologous cells of mammalian hosts to virus strains of feline leukemia virus (FeLV) are described. Cultures of feline embryo fibroblasts (FEF) were used in propagation assays. Embryo-derived cultures were used between the second and tenth *in vitro* passage levels. Replication of FeLV in homologous and heterologous cultures was determined by examination for development of group-specific antigen of the virus of the feline leukemia-sarcoma group (COCAL test). Inability to infect was defined if 10^3 infectous units produced productive infection of homologous FEF within 21 days after inoculation but failed to cause a detectable infection in heterologous culture on serial propagation over 42 days. Degree of susceptibility was determined in heterologous cells and homologous FEF cultures by a complement fixation antigen induction test. Selected strains of feline leukemia and sarcoma viruses with subgroups A, B, and C showed a different pattern in their ability to cross species barrier. Subgroup A had the narrowest host range infecting only cat and dog cells. Subgroup B had the widest range infecting the dog, human, monkey, bovine, pig and hamster species. Dog cells were 1,000-fold less sensitive than cat cells to the Gardener (CT-7) strain of Subgroup A virus. The susceptibility was equal to infection by clone-purified ST-FeLV of subgroup B. Guinea pig and cat cells displayed the same degree of sensitivity as cat cells to infection with clone-purified FL-237 strain of FeLV of subgroup C. In every case where purified FeLV of subgroups A, B, or C caused virus infection in a heterologous host species, there was no host range modification of virus due to loss in viral infectivity for the homologous host cells. Minor envelope antigenic differences within members of a subgroup may exist to account for differences in host range.

2017 SPONTANEOUS REGRESSION OF FRIEND VIRUS INDUCED LEUKEMIA: COINFECTION WITH REGRESSING AND CONVENTIONAL STRAINS OF VIRUS. (Eng.) Furmanski, P. (Michigan Cancer Found., Detroit); Baldwin, J.; Clymer, R.; Rich, M. A. *Science* 187(4172): 72-23; 1975.

I.p. inoculation of random-bred ICR/Ha Swiss weanling mice with mixtures of conventional Friend virus (CFV) and the regressing Friend virus strain (RFV) resulted in a very significant incidence of spontaneous regression of the leukemia induced by CFV. The incidence of regression varied from 22% in 10 mice injected with 10^{-2} CFV and 10^{-7} RFV to 30% in the 10 mice which received 10^{-2} CFV and 10^{-3} RFV. Linear regression analysis showed that the regression depended only on a threshold dose of RFV. The minimum amount of RFV which induced regression of CFV leukemia was below the titer for induction of Friend disease but correlated with the titer of lymphocytic leukemia (helper) activity in these stocks.

2018 PURINE METABOLISM IN MURINE VIRUS-INDUCED ERYTHROLEUKEMIC CELLS DURING DIFFERENTIATION *IN VITRO*. (Eng.) Reem, G. H. (New York Univ. Sch. Med., N.Y.); Friend, C. *Proc. Natl. Acad. Sci. U.S.A.* 72(4):1630-1634; 1975.

The activities of a number of enzymes in purine metabolism were studied with reference to controlling and differentiating erythroleukemic cells in tissue culture. The activities of four enzymes were studied in control clone line 745A of Friend erythroleukemia, and in those treated with 2% (vol/vol) of dimethylsulfoxide (Me_2SO); phosphoribosylpyrophosphate amidotransferase (PRibPP); ribose-5-phosphate aminotransferase, Hypoxanthine guanine phosphoribosyltransferase (HGPRT); or adenine phosphoribosyltransferase (APRT). Cytidine deaminase activity was assayed in cell-free extract by measuring uridine and uracil production from [^{14}C]cytidine. APRT activity was assayed by measuring AMP synthesis from [^{14}C]adenine using thin-layer chromatography on polyethylenimine plastic-backed plates. HGPRT activity was assayed by determining IMP production from [^{14}C]hypoxanthine. There was a significant drop in PRibPP activity from 6.3 in controls to 1.5 nmol/min/mg protein in Me_2SO-treated cells after 72 hr incubation. There was no significant change prior to 72 hr. When ammonia was substituted for glutamine in determining PRibPP activity, there was a comparable difference in specific activities between control and treated cells. The presence or absence of fresh medium did not alter the absolute difference in enzyme activity. The specific activities of HGPRT and APRT were not significantly affected by Me_2SO treatment. The Me_2SO treatment also did not significantly alter the ribonucleotide

synthesis by either adenine or hypoxanthine additions. Cytidine deaminase activity was lower after 48 hr in control cultures, but after 96 hr there was a 3-fold increase (from 0.46-1.80 nmol/min/mg protein). This rise in activity was in contrast to the fall of PRibPP synthesis. The data provide an example of repression and derepression of purine metabolism enzymes in mammalian cells *in vitro*.

2019 DIRECT TRANSFORMATION OF 3T3 CELLS BY ABELSON MURINE LEUKAEMIA VIRUS. (Eng.) Scher, C. D. (Child. Hosp. Med. Cent., Boston, Mass.); Sieger, R. *Nature* 253(5494):729-731; 1975.

A quantitative transformation assay is described. It involves the direct transformation of NIH/3T3 cells by the Abelson murine leukemia virus (MLV-A), which is potentially defective for virus replication. Cell-free filtrates of Abelson tumor extracts from BALB/c or Swiss mice consistently transformed NIH/3T3 cells. Transformed foci appeared 3-5 days after inoculation. Clones of transformed cells had saturation densities 3-6 times greater than those of untransformed NIH/3T3 cells. Simultaneous infections of NIH/3T3 and BALB/c-3T3 cells were made with serial dilutions of virus stock. A 2-fold difference in the frequency of transformation in the two cell lines indicated an NB-tropic transforming activity. The replicating Moloney virus (MLV-M) titer in the stock, detected by the XC cell test, was almost identical on N and B cells and was higher than that of transforming activity. The number of transformed foci was inversely proportional to virus dilution. Anti-MLV-M serum inhibited the transforming activity of virus stock 500-fold and reduced the number of XC plaques 40-fold. The endpoint dilution method was used to determine whether or not leukemogenic activity correlated with transforming activity. Only animals inoculated with virus prepared from transformed cells developed Abelson leukemia at one month postinoculation. Of the animals inoculated with virus from untransformed culture that gave positive XC cell test, 80% developed the thymic tumors of the Moloney leukemia after three months. A clone of virus was used to confirm the transforming activity of MLV-A. It is suggested that this activity is necessary in the induction of the murine B-cell leukemia.

2020 BIOSYNTHESIS OF RAUSCHER LEUKEMIA VIRAL PROTEINS. (Eng.) Naso, R. B. (Univ. Texas Syst. Cancer Cent. M. D. Anderson Hosp. Tumor Inst., Houston); Arcement, L. J.; Arlinghaus, R. B. *Cell* 4(1):31-36; 1975.

The *in vivo* synthesis and subsequent cleavage of Rauscher leukemia virus (RLV)-specific, intracellular polyproteins is described. Antisera to disrupt RLV were used to precipitate viral polypeptides from extracts of infected NIH Swiss mouse cells. These labeled [^{3}H] RLV proteins ranged in molecular weight of 10,000-30,000 daltons with the 14,000-30,000 size comprising 75% of the total RLV protein. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) identified three distinct viral glycoproteins. Three of four distinct virus-specific glycosylated polypeptides comigrated with the glycoproteins of mature virons. Cytoplasmic extracts, pulse-labeled for 10-20 min with ^{35}S-methionine, did not reveal any nonglycosylated internal polyproteins of mature virons. Cyclohexamide (100 μg/ml) did not significantly alter the pattern of conversion of radioactivity during the chase from large polypeptides to those corresponding to mature protein. This indicates that conversion did not require *de novo* synthesis. Based on these pulse-chase experiments, the hypothesis is supported that the 35S viral RNA subunit is translated into primary gene products with the properties of precursor polypeptides.

2021 REPLICATION KINETICS OF N- AND B-TROPIC MURINE LEUKEMIA VIRUSES ON PERMISSIVE AND NONPERMISSIVE CELLS *IN VITRO*. (Eng.) Declève, A. (Stanford Univ. Sch. Med., Calif.); Niwa, O.; Gelmann, E.; Kaplan, H. S. *Virology* 65(2):320-332; 1975.

The kinetics of infection by three different MuLV viruses is described; the viruses included radiation leukemia virus (RadLV), Gross-AKR leukemia virus (GLV), and Moloney leukemia virus (MLV). Cultures of the Fv-1 genotype were inoculated with 0.4 ml virus preparation. Three assay procedures were used: (1) direct plaque assay, (2) reverse XC cell plaque procedure, and (3) MuLV immunofluorescence. Virus titrations were performed three days after infection. A single virus particle that produced infection gave a "one-hit" curve. When two viral particles were required the titration curve was referred to as a "two-hit" curve. Titration patterns of test viruses revealed that (1) host restriction of MuLV replication is determined by the slope of the titration curve (one-hit or two-hit) and (2) the level of sensitivity is determined by the relative positions of the curves and end point dilution titers. The results support a two-hit type response to N- or B-tropic MuLV infection by cells of heterologous Fv-1 genotype. The Fv-1 gene regulates intracellular repression or promotion of viral replication and does not code for a cell-surface receptor for, or barrier to, infection by MuLV. Virus spread is by cell-to-cell contact.

2022 STUDIES ON REVERSE TRANSCRIPTASE OF RNA TUMOR VIRUSES. III. PROPERTIES OF PURIFIED MOLONEY MURINE LEUKEMIA VIRUS DNA POLYMERASE AND ASSOCIATED RNase H. (Eng.) Verma, I. M. (Tumor Virol. Lab., Salk Inst., San Diego, Calif.). *J. Virol.* 15(4):843-854; 1975.

The presence of the nuclease activity that can selectively degrade the RNA moiety of an RNA-DNA hybrid (RNase H) in purified Moloney murine leukemia virus (M-MuLV) DNA polymerase was studied, and the properties of this DNA polymerase were compared with purified DNA polymerase from avian myeloblastosis virus (AMV). Polyacrylamide gel electrophoresis and sedimentation on glycerol gradients showed the molecular weight of M-MuLV to be 80,000. A compari-

son of transcription of various polyribonucleotides and polydeoxyribonucleotides of M-MuLV and AMV DNA polymerase showed that both enzymes were able to transcribe a variety of templates with equal efficiency, except for native viral 70S RNA without exogenous primer. Addition of oligo(dT) enhanced the transcription of 70S viral RNA 5- to 10-fold by M-MuLV DNA polymerase. The RNase H activity of purified M-MuLV DNA polymerase was assayed by using the ϕX174 DNA-[^{3}H]RNA hybrid as substrate. The RNA moiety of this hybrid was susceptible to RNase H activity only in RNA-DNA hybrid form. The ratio of DNA polymerase to RNase H activity of the two enzymes was not significantly different. In determining the mode of action of RNase H, it was shown that the M-MuLV DNA polymerase-associated RNase H acts as a random exonuclease. It is concluded that this RNase H cleaves at the 3' side of the 3', 5'-phosphodiester bond to yield products containing 5'-phosphate and 3'-OH ends. The average size of the digestion product was 15-20 nucleotides, compared to the average size of the AMV DNA polymerase digestion product of 10-12 nucleotides.

2023 SEROLOGICAL STUDIES WITH LOW-MOLECULAR-WEIGHT POLYPEPTIDES FROM THE MOLONEY STRAIN OF MURINE LEUKEMIA VIRUS. (Eng.) Parks, W. P. (Natl. Cancer Inst., Bethésda, Md.); Noon, M. C.; Gilden, R.; Scolnick, E. M. *J. Virol.* 15(6):1385-1395; 1975.

Major virion low-molecular-wt polypeptides isolated from the Moloney strain of murine leukemia virus (type C) were studied for their type-specific reactivities. The polypeptides were isolated by agarose chromatography in 6 M guanidine hydrochloride. Elution volume and relative mobility in sodium dodecyl sulfate-polyacrylamide gels showed the polypeptides to have molecular weights of 15,000 (p15), 12,000 (p12), and 10,000 (p10). Each polypeptide could be iodinated and employed in double antibody radioimmunoassay procedures. All three polypeptides demonstrated a high degree of type specificity in serologic immunoprecipitation analysis and in corresponding competition immunoassays. The p15 was immunologically distinct from other virion polypeptides including p12 and p10; the p12 and p10 were highly related to each other but not to other virion polypeptides and were even more type specific than the p15 in serologic tests. Competition immunoassays with p15 and p10 indicated that the Moloney strain of MuLV is only a distant relative of the Friend-Rauscher group. The combined use of the Kirsten and Moloney low-molecular-wt polypeptide immunoassays suggest that xenotropic viruses constitute yet another group(s) of murine leukemia virus with distinct type specific antigens, in the heterogeneous group of mouse type C viruses.

2024 PURIFICATION AND CHARACTERIZATION OF THE DNA POLYMERASE AND RNASE H ACTIVITIES IN MOLONEY MURINE SARCOMA-LEUKEMIA VIRUS. (Eng.) Gerard, G. F. (St. Louis Univ. Sch. Med., Mo.); Grandgenett, D. P. *J. Virol.* 15(4):785-797; 1975.

Two RNase H (RNA-DNA hybrid ribonucleotidohydrolase, EC 3.1.4.34) were identified in lysates of Moloney murine sarcoma-leukemia virus (MSV) produced by the transformed rat cell line 78A-1. The two RNase activities observed in MSV appeared to represent distinctly different enzymes. The larger enzyme (RNase H-I, represented about 10% of the RNase H activity in the virion. RNase H-I had a sedimentation coefficient of 4.4S (apparent molecular wt 70,000), whereas the smaller enzyme (RNase HII) sedimented at 2.6S (30,000 molecular wt). RNase H-I required Mn^{2+} (2 mM optimum) for activity with a [^{3}H]poly(A) x poly(dT) substrate, while RNase H-II preferred Mg^{2+} (10-15 mM optimum) 2.5-fold for the degradation of the substrate. RNase H-I degraded [^{3}H]poly(A) x poly(dT) and [^{3}H] poly(C) x poly(dG) at approximately equal rates, whereas RNase H-II degraded the former substrate six and 60 times faster than the latter substrate in the presence of Mn^{2+} and Mg^{2+}, respectively. Whereas RNase H-I copurified with MSV DNA polymerase, RNase H-II did not have associated DNA polymerase activity, and behaved as a single enzyme species. MSV DNA polymerase (RNase H-I), purified by Sephadex G-100 gel filtration followed by phosphocellulose, poly(A) x oligo(dT)-cellulose, and *O*-(diethylaminoethyl) cellulose chromatography, transcribed heteropolymeric regions of avian myeloblastosis virus 70S RNA at a rate comparable to avian myeloblastosis virus DNA polymerase purified by the same procedure. The author cites evidence indicating that RNA-directed DNA polymerase and RNase H activity reside on the same polypeptide subunit in avian RNA tumor viruses.

2025 STUDIES OF FBJ OSTEOSARCOMA VIRUS IN TISSUE CULTURE. II. AUTOINHIBITION OF FOCUS FORMATION. *(Eng.)* Levy, J. A. (Natl. Cancer Inst., Bethesda, Md.); Hartley, J. W.; Rowe, W. P.; Huebner, R. J. *J. Natl. Cancer Inst.* 54(3): 615-619; 1975.

The prozone or autoinhibitory effect of murine sarcoma virus (FBJ-MuSV) was compared to the inhibitory effect of nonfocus-forming murine leukemia virus (FBJ-MuLV). MuLV was detected by the XC plaque assay and MuSV was detected by focus formation assay. The Harvey MuSV (H-MuSV) and Moloney MuSV (M-MuSV) were grown in NIH Swiss mouse embryo (NIH-ME) cells. Focus formation inhibition occurred when virus preparations were tested undiluted or diluted up to 1:4. At a 1:10 dilution of MuSV the kinetics of focus formation became one-hit. FBJ preparations had an excess of 3-4 $\log_{10}$ of MuLV, suggesting that focus formation by high concentrations of FBJ-MuSV is inhibited by this excess of FBJ-MuLV. The difference in extent of inhibition by the various cell lines was observed with no additive inhibition occuring when the undiluted MuSV preparation was taken in presence of added MuLV. Dilution of H-MuSV and M-MuSV in the absence of added MuLV gave 2-3 times as many foci as undiluted FBJ virus containing the same number of MuSV particles showing the autoinhibitory effect of MuSV system. The production of progeny of MuSV varied directly with the number and size of the foci. The effect of the ratio of MuLV to MuSV on focus formation was that a multiplicity of infection (MOI) of 1-10 of MuLV/cell significantly in-

hibited focus formation in ME cells. A low MOI (0.1-0.01) enhanced focus formation. This inhibition was not due to interferon, because it was previously indicated that RNA tumor virus infection does not induce detectable amounts of interferon. The FBJ MuLV-MuSV complex may be a model system for the detection of sarcoma viruses in spontaneous tumors in animals where inhibition by excess non-transforming virus could be an important phenomenon.

026 SEPARATION OF SARCOMA VIRUS-SPECIFIC AND LEUKEMIA VIRUS-SPECIFIC GENETIC SEQUENCES F MOLONEY SARCOMA VIRUS. (Eng.) Scolnick, E. M. Natl. Cancer Inst., Bethesda, Md.); Howk, R. S.; nisowicz, A.; Peebles, P. T.; Scher, C. D.; Parks, . P. *Proc. Natl. Acad. Sci. USA* 72(11):4650-4654; 975.

he nucleic acid sequences in nonproducer cells ransformed by two types of replication-defective, NA-containing, viruses isolated by passage of oloney leukemia virus in BALB/c mice (i.e., Moloney arcoma virus and Abelson leukemia virus) were stuied. DNA probes from the Moloney leukemia virus etected RNA in both Abelson virus-transformed nonroducer cells and Moloney sarcoma virus-transformed onproducer cells. A sarcoma-specific complementary NA, prepared from the Moloney sarcoma virus, had xtensive homology to RNA found in heterologous onproducer cells transformed by Moloney sarcoma irus, had little homology to RNA in cells producing oloney leukemia virus, and no detectable homology o RNA in nonproducer cells transformed by the belson virus. By analogy to earlier data on avian nd mammalian sarcoma viruses, these results suggest hat the Moloney sarcoma virus arose by recombination between a portion of the Moloney leukemia virus enome and additional sarcoma-specific information, nd indicate that the expression of this information s not essential for Abelson virus-mediated fibrolast transformation.

)27 SARCOMA-NEGATIVE LEUKEMIA-POSITIVE TRANSFORMED CELL CULTURE ESTABLISHED FROM A JRINE SARCOMA VIRUS-INDUCED RAT BONE TUMOR. (Eng.) ian, J. C. (Univ. Texas System Cancer Center M. D. nderson Hosp. and Tumor Inst., Houston, Tex. 77025); ist, J. L.; Dmochowski, L. *Cancer Res.* 35(9):2475-481; 1975.

culture established from a murine sarcoma virus SV)-induced rat bone tumor is described with reect to its retention, after more than 130 *in vitro* assages, of: refractile round cell morphology, e ability to form colonies in agar, and enhanced ucose uptake. Two separate cell cultures were tablished from two individual MSV-Soehner-Dmoowski isolate (MSV-SD)-induced NB rat bone tuors. Cells of one bone tumor culture (RBT-E) are early *in vitro* passages. These cells formed colonies in agar medium and took up 2-deoxy-D-[^{3}H]glucose at a rate five times that of normal nontransformed rat embryo cells. Cells of the RBT-E culture released both MSV and murine leukemia virus (MuLV) and therefore contain sarcoma-positive leukemia-positive transformed cells. The other rat bone tumor culture (RBT-L) produced MSV at early passages. Cells of the RBT-L culture formed colonies in agar medium and took up 2-deoxy-D-[^{3}H]glucose at a rate three times that of rat embryo cells, indicating the presence of transformed cells within the RBT-L culture. However, cells of the RBT-L culture at late passages produced only MuLV and no detectable MSV activity (as shown by the lack of tumor-inducing activity and the lack of focus-forming activities by direct assay or by infectious center assay). Attempts to rescue MSV activity from RBT-L cells by cocultivation with MuLV-producing mouse cells were not successful. The MuLV found in the RBT-L cells, however, was found to be a competent helper virus capable of rescuing the MSV genome from MSV-SD-induced hamster bone tumor cells. All the available evidence supports the notion that late passages of the RBT-L culture contain transformed cells that do not produce conventionally detectable MSV. These cells are referred to as sarcoma-negative leukemia-positive cells. The sarcoma-negative leukemia-positive cells represent a different kind of MSV-induced transformed cells and provide a unique system for studies in search of MSV markers such as MSV-specific antigens and MSV-specific nucleotide sequences.

2028 GLUCOCORTICOID-RECEPTOR INTERACTION AND INDUCTION OF MURINE MAMMARY TUMOR VIRUS. *(Eng.)* Young, H. A. (Natl. Cancer Inst., Bethesda, Md.); Scolnick, E. M.; Parks, W. P. *J. Biol. Chem.* 250(9):3337-3343; 1975.

The role of cytoplasmic receptors in the glucocorticoid induction of murine mammary tumor virus (MMTV) was analyzed in terms of specific binding and structure-activity relationships. S100 cytoplasmic extracts from the mouse mammary tumor cell line, C3H MT clone 6 were used for characterization of the glucocorticoid receptor. In the presence of varying concentrations of ^{3}H-dexamethasone, saturation of the binding sites occurred at 4 x 10^{-8} to 8 x 10^{-8}M with one class of receptor present. Dexamethasone, corticosterone and progesterone were comparitively effective in competing for hormone receptor. Dexamethasone (1 x 10^{-10}M) was the most potent inducer of MMTV supernatant DNA polymerase. Negative results were obtained with progesterone and other steroids. Levels of nonradioactive hormone that blocked ^{3}H-dexamethasone uptake in tissue culture closely paralleled levels necessary to inhibit cytoplasmic receptor binding. Cells which demonstrated markedly different MMTV phenotypic expression contained comparable levels of receptor. Increased amounts of dexamethasone-receptor complex were bound to nuclei with a plateau reached after 60 min of "activation." The major physiologic determinants of all these events appear to be hormone levels, receptor levels, and transcription of the target cell chromatin.

2029 THE MORPHOLOGY OF MURINE ONCORNAVIRUSES FOLLOWING DIFFERENT METHODS OF PREPARATION FOR ELECTRON MICROSCOPY. (Eng.) Sarkar, N. H. (Inst. Med. Res., Camden, N.J.); Manthey, W. J.; Sheffield, J. B. *Cancer Res.* 35(3):740-749; 1975.

The effects of various preparative procedures in neutralizing the effects of osmotic pressure and surface tension for murine leukemia and mammary tumor viruses were studied by electron microscopy. Fixing in glutaraldehyde, gradual dehydration in alcohol and water mixtures followed by air-drying from alcohol, and critical-point drying were the techniques studied, both alone and in combination. Metal shadowing techniques were used to analyze the three-dimensional aspects of particle morphology. Both the tumor and leukemia viruses appeared in head-and-tail forms, with a peak head diameter of 122 and 130 nm, respectively, using conventional negative staining procedures with neutral sodium phosphotungstate. Round virions with peak diameters of 148 and 130 nm, respectively, were obtained with negative staining with uranyl acetate. Virus prefixed in uranyl acetate gave peak diameters of 143 and 123 nm. A combination of glutaraldehyde fixation, gradual dehydration, and critical-point drying in CO_2 preserved the spherical shape of viruses and produced peak diameters of 99 and 82 nm, respectively. Except under conditions of negative staining with phosphotungstate, the mammary tumor virus was always larger than the leukemia virus. Metal shadowing of virus particles showed flattening, except with critical-point drying. No shadow was made by negatively-stained particles. Alterations in osmotic strength from hypotonic to isotonic sucrose solutions enabled conversion of virus from spherical to head-and-tail forms. It is concluded that a combination of all three treatments, preferably with uranyl acetate staining, yields spherical virus morphology with minimum distortion.

2030 INHIBITION OF RNA-DEPENDENT DNA POLYMERASE OF ONCORNA VIRUSES BY CARBOPOL 934. (Eng.) Bloemers, H. P. J. (Dep. Biochem., Univ. Nijmegen, Netherlands); Van Der Horst, A. *FEBS Lett.* 52(1): 141-144; 1975.

RNA-dependent DNA polymerase (reverse transcriptase) activity of Rauscher murine leukemia virus (R-MuLV) and avian myoblastosis virus (AMV) was studied after exposure to Carbopol 934, a cross-linked polyacrylic acid (molecular weight 3×10^6). Enzyme activity with poly A and poly C templates and endogenous activity were assayed with (^{3}H)-dTTP or (^{3}H)-dGTP (20 μM and 300 Ci/M) labeling and counting on a liquid d scintillation spectrometer. Carbopol concentrations of 50 μg/ml-500 μg/ml were severly inhibitory to R-MuLV reverse transcriptase activity. Strong inhibition of AMV enzyme activity was observed at 100 μg/ml Carbopol and higher. Endogenous activity of R-MuLV enzyme was slightly stimulated at 1 and 5 μg/ml Carbopol, but higher concentrations were inhibitory. When Carbopol (40 μg/ml) in saline was added to R-MuLV samples before incubation at 56 C, the enzyme showed greater resistance to heat denaturation than did controls. A Lineweaver-Burk plot of AMV reverse transcriptase showed competitive-type inhibition indicating that the negatively charged polymer mimics nucleic acids, thereby interfering with template-enzyme binding. Carbopol may be useful in discriminating two causes of tumor growth: viral infection of uninfected cells and cell division of persistently infected cells.

2031 *IN VITRO* POLYOMA DNA SYNTHESIS: SELF- ANNEALING PROPERTIES OF SHORT DNA CHAINS. (Eng.) Francke, B. (Salk Inst., P.O. Box 1809, San Diego, Calif. 92112); Vogt, M. *Cell* 5(2):205-211; 1975.

The self-annealing of short DNA chains was studied, and potential complication of short chain DNA intermediate purification was investigated. Pulse-labeled BudR subsituted short DNA chains were prepared from polyoma replicating intermediates. Kinetic studies revealed some degree of complementarity, illustrated by 28% resistance to S_1 endonuclease after self-annealing to plateau levels. The self-annealing properties were not found dependent on the type of *in vitro* system, while a semi-discontinuous mode of chain growth was experimentally supported. The self-annealing of the faster sedimenting portion of short DNA chains was higher than that of the slower sedimenting portion, indicating continuously growing strands from the other side of the fork. Cleavage by the restriction endonuclease from *Hemophilus parainfluenzae* (HpaII) suggested the presence of multiple origins of replication. The results indicate that polyoma DNA synthesis proceeds semi-discontinuously, rather than totally discontinuously; however, it can generate self-complementary short chains.

2032 INTERACTIONS OF POLYOMA AND MOUSE DNAs III. MECHANISM OF POLYOMA PSEUDOVIRION FORMATION. (Eng.) Turler, H. (Dept. Mol. Biol., Univ. Geneva, Switzerland). *J. Virol.* 15(5):1158-1167; 1975.

The events leading to virion and pseudovirion replication were studied in primary mouse kidney cell cultures infected with polyoma virus. The confluent cultures were infected with virus and synchronized for the onset of cellular and viral DNA replication. The cellular and viral DNA were labeled with ^{3}H-thymidine, and the kinetics of DNA synthesis and the formation of low-molecular-weight fragmented mouse DNA (mouse f-DNA) were studied. DNA was determined by fluorimetry or the diphenylamine color reaction. The viral particles were harvested at various intervals after infection, and the proportion of pseudovirions was determined by sedimentation velocity analysis. The synthesis of cellular and viral DNA were closely coordinated both temporally and in terms of synthetic rate. The formation of mouse f-DNA was detected several hours after the onset of mouse- and polyoma-DNA replication, its formation coinciding in time with the appearance of progeny virus. The proportion of pseudovirions formed appeared to be inversely related to the amount of viral DNA synthesized and was independent of the formation of mouse f-DNA. When DNA synthesis was partially inhibited

y mitomycin C, there was a 2- to 3-fold increase
n the proportion of pseudovirions obtained compared
ith that from untreated cultures. The proportion of
seudovirions was always lower in the supernatant
edium than in the remaining monolayer, and the pro-
ortion increased slightly with time after infection.
hus, polyoma virus preparations with a low (10-20%)
r high (60-80%) proportion of pseudovirions can be
btained. It appears that pseudovirions are an ac-
idental by-product of virus formation, and that
ragments of host DNA compete with viral DNA for en-
apsidation.

2033 SPECIFIC INCREASE IN POLYAMINE LEVELS IN CHICK EMBRYO CELLS TRANSFORMED BY ROUS ARCOMA VIRUS. (Eng.) Don, S. (Hebrew Univ. Hadassah ed. Sch., Jerusalem, Israel); Wiener, H.; Bachrach, . *Cancer Res.* 35(1):194-198; 1975.

o demonstrate the relation between cellular poly-
mines and transformation with oncogenic viruses,
hick embryo fibroblasts and chorioallantoic mem-
ranes were infected with wild type (SR-17A) or tem-
erature-sensitive (T5) Rous sarcoma virus (RSV)
utants at permissive (37 C) or nonpermissive (42 C)
emperature. Cells were analyzed for polyamines,
nd the results were compared to polyamine content
f chorioallantoic membranes infected with nononco-
enic viruses (influenza strain PR8, Newcastle dis-
ase velogenic strain WR). Primary chick embryo
ibroblasts were infected with RSV (0.2 ml) on day 1.
ubcultures were made on day 5, and secondary cells
ere infected with RSV (1.0 ml) on day 6. Growth
edium was first changed at least three days after
ells were seeded and once during each subsequent
ay. The incubation temperature of some cultures
as shifted on day 10. All groups were analyzed for
olyamines on day 11. The chorioallantoic membranes
f 12- to 13-day-old chick embryos were infected
ith RSV (0.05-0.1 ml). Membrane fragments were
ollected six days later, washed in 0.85% NaCl, and
nalyzed for polyamines. Only putrescine accumulated
n chick embryo fibroblasts transformed by RSV at
7 C. The nononcogenic viruses had no effect on the
olyamine content of chorioallantoic membranes.
ransformation of membranes by both the SR-17A and
5 strains of RSV at 37 C caused a 2- to 4-fold
ncrease in cellular spermidine and putrescine con-
ent. At 42 C, RSV strain T5 did not alter cellular
orphology or polyamine content. These data suggest
hat if polyamines are involved in neoplastic growth,
hen polyamine analogs may present a new series of
otential therapeutic agents.

034 A QUANTITATIVE AUTORADIOGRAPHIC STUDY OF NUCLEOLUS-ASSOCIATED RNA AND DNA SYNTHESIS URING THE ECLIPSE PHASE IN ROUS SARCOMA VIRUS-IN- ECTED CHICKEN FIBROBLASTS. (Eng.) Suskind, R. G. Natl. Cancer Inst., Bethesda, Md.); Michelson-Fiske, .; Haguenau, F.; Rabotti, G. F. *J. Natl. Cancer nst.* 54(2):349-360; 1975.

unctional and morphological differences between the ensitivity of nucleoli of Rous sarcoma virus (RSV)- ransformed cells and that of newly-infected cells to the action of actinomycin D were examined by quantitative light and electron microscope autoradiography. Third passage or subsequent cultures of chick embryo fibroblasts were infected with the Schmidt-Ruppin strain of RSV (SR-RSV) at multiplicity of infectivity of 10. Control cells were exposed to virus suspension heat-inactivated at 90 C for 20 min. Actinomycin D was used at a concentration of 0.2 μg/ml. Cells were pulse-labeled with tritiated uridine or tritiated actinomycin D. Nucleoli of cells with a high incidence of transformation (2-3 passages) were compared with those of cells newly infected. There was an initial increase in RNA synthesis in transformed over newly infected nucleoli, and a continuous incremental RNA synthesis during recovery from inhibition by actinomycin D which was parallel in transformed and newly infected cells. Application of statistical confidence belts for binomial proportions to the segregation effect of the drug on nucleolar ultrastructure showed a significant increase in the proportion of morphologically recovered nucleoli in transformed cells compared to newly infected cells. Those differences observed in transformed cells were induced within 80 min. Quantitative electron microscope autoradiography showed a 3- to 4-fold majority of the label over the fibrillar portion of the nucleolus in both infected and noninfected cells. Cells were labeled with 0.6 μCi [^{3}H]actinomycin D/ml for ten minutes to three hours. After a chase period of ten hours, the retention of the drug as measured by total nuclear grain count, and relative distribution in infected cells was twice that of noninfected cell nucleoli. A concomitant redistribution of intranuclear and cytoplasmic DNA label was also seen. These data indicate a virus-induced amplification of actinomycin D binding sites in nucleolar chromatin.

2035 TRANSFORMATION BY A TEMPERATURE SENSITIVE MUTANT OF ROUS SARCOMA VIRUS IN THE ABSENCE OF SERUM. (Eng.) Bell, J. G. (Imp. Cancer Res. Fund Lab., London, England); Wyke, J. A.; MacPherson, I. A. *J. Gen. Virol.* 27(2):127-134; 1975.

The relationship between events in the cell cycle and the continued expression of virus functions was studied by subjecting phenotypically normal chicken embryo fibroblasts infected with the temperature-sensitive transformation mutant of Rous sarcoma virus, *tsLA*24PR-A, to serum depletion at nonpermissive temperatures. The permissive temperature of this virus is 35 C; the restrictive temperature is 41 C. A two-day exposure of *LA*24-infected cultures to serum-free medium rendered cells stationary. The percentage of ^{3}H-thymidine-labeled nuclei in cultures treated with colchicine (16%) and in control cultures which did not receive the drug (14%) was similar, indicating that the cells had been arrested after mitosis and prior to complete DNA synthesis. The percentage of labeled nuclei showed a parallel increase to ^{3}H-thymidine incorporation, suggesting that the cells were in the S phase of the cell cycle. Cultures kept in serum-free medium showed a 10-fold decrease in virus titer. Temperature shift alone induced cells to incorporate thymidine to a level of serum-free cells. This increase in thymidine incorporation

in serum-free medium occurred less rapidly than did the increase when serum-containing medium was added at the time of shift. These data suggest that passage of the cells through their growth cycle, in the absence of serum factors needed for growth of untransformed cells, is related to the exposure of the virus to transforming function.

2036 RNA-DEPENDENT DNA POLYMERASE ACTIVITY OF RNA TUMOR VIRUSES. V. ROUS SARCOMA VIRUS SINGLE-STRANDED RNA-DNA COVALENT HYBRIDS IN INFECTED CHICKEN EMBRYO FIBROBLAST CELLS. (Eng.) Leis, J. (Duke Univ. Med. Cent., Durham, N.C.); Schincariol, A.; Ishizaki, R.; Hurwitz, J. *J. Virol.* 15(3):484-489; 1975.

The characteristics of isolated RNA-DNA covalent hybrid molecules from Rous sarcoma virus (RSV)-infected chick embryo fibroblast cells were examined. A temperature-sensitive RSV mutant, LA335, was used to demonstrate that the appearance of these hybrid molecules in nuclear cell fractions requires a functional reverse transcriptase. Nucleic and cytoplasmic fractions were prepared from fibroblast cells infected with [^{3}H]uridine-labeled RSV. Fibroblast cells (1.2 x 10^9) were infected with tritium-labeled RSV at an infection multiplicity of 1. Of label recovered in the cytoplasmic fraction, 90% banded at the density of free RNA in Cs_2SO_4 after heat treatment at 100 C for three minutes. Material was isolated from the nuclei six hours after infection, treated with 3% formaldehyde for 15 min at 65 C, and subjected to isopycnic Cs_2SO_4 gradient centrifugation. Of the total tritium recovered, 33% banded in the RNA region, 21% banded in the hybrid region, and the remainder banded in the DNA region. The molecular weight of the largest RSV RNA-DNA covalent hybrid structures isolated from nuclei as measured in dimethyl sulfoxide (80%) sucrose gradients was approximately 10^6 to 2.5 x 10^6. Experiments on the presence of viral [^{3}H]RNA-DNA covalent hybrid in RSV LA335-infected cells showed that the formation of single-stranded viral RNA-DNA covalent hybrid molecules requires reverse transcriptase.

2037 A REPLICATION DEFECTIVE MUTANT OF ROUS SARCOMA VIRUS WHICH FAILS TO MAKE A FUNCTIONAL REVERSE TRANSCRIPTASE. (Eng.) Friis, R. R. (Sch. Med., Univ. South. Calif., Los Angeles); Mason, W. S.; Chen, Y. C.; Halpern, M. S. *Virology* 64(1):49-62; 1975.

To identify the temperature-sensitive function of *ts* 672, the replication-defective temperature-sensitive mutant of Rous sarcoma virus, the large amounts of noninfectious viral particles (NI 672) that are produced at the nonpermissive temperature (41 C) were analyzed. This mutation was also compared to other known mutations affecting replication. Virus stocks were cloned by isolation of single foci from monolayers of chick helper factor negative cells of chick embryos from avian leukosis-free flocks and a Japanese quail cell line chronically infected with the Bryan high titer strain of Rous sarcoma virus. The only structural defect observed with NI 672 was the absence of the virion-associated RNA-dependent DNA polymerase activity characteristic of all infectious RNA tumor viruses. The DNA polymerase activity of the *ts* 672 was as temperature-stable as the wild type enzyme. Mixed infection tests with the Bryan high titer strain and the para-influenza virus type 1 strain Sendai/52, both DNA polymerase mutants, both failed to show complementation. Recombination studies with *ts* 672 and an avian leukosis virus (Rous sarcoma-associated virus type 6, subgroup B) showed a high degree of genetic linkage between the temperature-sensitive DNA polymerase function of *ts* 672 and the determinants for the host range of these viruses, the viral envelope proteins. It is suggested that the *ts* 672, and *ts* 335 and *ts* 337 (two temperature-sensitive mutants of the Prague strain of Rous sarcoma virus, subgroup C), may provide an excellent system for the investigation of the nature of RNA tumor virus recombination; they represent two readily distinguishable physiological groups, although they apparently all belong to the same cistron.

2038 TURNOVER OF HIGH-MOLECULAR-WEIGHT CELL SURFACE PROTEINS DURING GROWTH AND EXPRESSION OF MALIGNANT TRANSFORMATION. (Eng.) Rieber, M. (Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 101, Venezuela); Bacalao, J.; Alonso, G. *Cancer Res.* 35(8):2104-2108; 1975.

The turnover of cell surface proteins in normal rat kidney cells (NT_3KR) transformed by a temperature-sensitive Rous sarcoma virus was studied by polyacrylamide gel electrophoresis and autoradiography using cell monolayers prelabeled by lactoperoxidase-catalyzed radioiodination. Labeling of serum-starved cells under nonpermissive conditions for the expression of transformation reveals most of the radioactivity in the 250,000 molecular wt region. Parallel labeling of cells simultaneously exposed to serum limitation, under conditions that are permissive for the expression of transformation, revealed some radioactivity in the same slow-migrating region, but most of the label appears in the two faster migrating regions. When nonserum-starved cells were seeded in nonpermissive conditions and iodinated after 2.5 days, the iodination pattern revealed a great proportion of the iodination in fast migrating regions, as well as some significant radioactivity in the slower region. After 16 hr, there was a preferential decrease in the two slow-migrating bands, and an increase in most other components, apparently originating from turnover of the slow species. The results suggest that the high molecular wt surface components described are normally subject to a significant turnover which may be accelerated during growth of transformation; however, this may occur as part of a normal shedding mechanism.

2039 GENETIC CONTROL OF RESISTANCE OF CHICK EMBRYO CULTURES TO RSV (RAV 50). *(Eng.)* Pani, P. K. (Houghton Poult. Res. Stn., Huntingdon, England). *J. Gen. Virol.* 27(2):163-172; 1975.

The genetic control of resistance of chick embryo

cultures to Rous sarcoma virus (RSV)(RAV 50) was studied in crosses between Reaseheath lines, I, C, and W and test crosses between WC(F_1) and RPRL line 7. Sixty-four embryos of WC(F_2), 61 embryos of IW (F_2), 47 embryos of WC(F_1) x IW(F_1)(F_2), 39 embryos of WC(F_1) x C, four embryos of IW(F_1), 12 embryos of C line, 13 embryos of line 7, and 52 embryos of line 7 x WC(F_1) were cultured individually and information of phenotypic segregation of resistance and susceptability were obtained. Embryos with five or fewer foci were considered resistant. No embryo cultures infected with two viruses exceeded a focus level of five. In all three F_2 families, the segregation of C/O and C/BD phenotypes occurred, except in one embryo of C/B phenotype in the WC(F_2) family. In WC(F_1) x C and IW(F_1) x I back crosses all embryos cultured were of C/O phenotype. The observed segregation ratio (3:1) agreed well with the expected ratio, indicating that the response to subgroup B virus was controlled by one pair of genes. All embryos of the WC x C and IW x I back crosses were of C/O phenotype, showing that the susceptability gene for response to RSV(RAV 50) was controlled by WC(F_1) and IW(F_1) parents. In all three F_2 families the segregation of C/D phenotype occurred consistently. On the basis of two unlinked genes that controlled embryo response to virus, the X^2 analysis showed that the genes controlling the types of responses were linked. Therefore analysis of the segregation results of resistance and susceptibility in F_2, back crosses, and test crosses suggests that the tumor virus *b* genes pleiotropically control the resistance of the embryo cultures to RSV(RAV 50).

2040 A PRIMER RIBONUCLEIC ACID FOR INITIATION OF *IN VITRO* ROUS SARCOMA VIRUS DEOXYRIBONUCLEIC ACID SYNTHESIS. NUCLEOTIDE SEQUENCE AND AMINO ACID ACCEPTOR ACTIVITY. *(Eng.)* Harada, F. (Dep. Physiol. Chem., Univ. Wisconsin, Madison); Sawyer, R. C.; Dahlberg, J. E. *J. Biol. Chem.* 250 (9):3487-3497; 1975.

The nucleotide sequence is reported of the primer RNA required for the initiation of synthesis *in vitro* of DNA using the Rous sarcoma virus (RSV) viron-associated DNA polymerase. Cellular spot 1 RNA was purified by two dimensional gel electrophoresis from RNA of chicken embryo fibroblast cells grown in tissue culture. Oligonucleotides from RNase T_1 or pancreatic RNase digestion were separated by two dimensional paper electrophoresis. This allowed the assignment of individual oligonucleotides to the 5' or 3' portion of the molecule. Individual oligonucleotides were eluted and further digested with various ribonucleases. Each redigestion product was identified by its mobility relative to standards. A 75 nucleotide long sequence was deduced which could be folded into a cloverleaf structure similar to transfer RNA (tRNA). The anticodon loop contained the sequence for tryptophan. The tRNA character of this molecule was confirmed by aminoacylation studies using nonradioactive spot 1 RNA from chicken liver. Spot 1 RNA contained a number of sequence characteristics not usually found in tRNA. The author proposes three reasons as to why tRNA would be used as a primer for RSV DNA synthesis: (1) the RNA may have special features facilitating a dual function, (2) it may be advantageous for the virus to use cellular molecules with sequences conserved either within cells or between species, or (3) by the change occurrence of RNA having a sufficient sequence complementarity to 35S RNA of RSV to allow priming of DNA synthesis.

2041 THE MOLECULAR GENETICS OF HUMAN CANCER AND ITS ETIOLOGIC IMPLICATIONS. (Eng.) Spiegelman, S. (Coll. Physicians and Surgeons, Columbia Univ., New York, N.Y. 10032); Axel, R.; Baxt, W.; Kufe, D.; Schlom, J. *Genetics* 79(Suppl.):317-338; 1975.

The genotypic change underlying the cellular transformation to malignancy was studied using the avian myeloblastosis virus (AMV), Rous sarcoma virus (RSV), murine leukemia virus (MuLV), murine sarcoma virus (MuSV), and murine mammary tumor virus (MMTV). DNA-RNA hybridization experiments were performed to determine whether human tumors contain viral-related RNAs. The RNA from human breast cancer cells formed complexes with MMTV viral-specific ^{3}HDNA in 67% of the cases studied, while RLV viral-specific DNA formed complexes with up to 92% of the human leukemia, sarcoma, and lymphoma RNAs. The specificity pattern of the unique RNA found in the human neoplasias was also in complete agreement with what has been described for the corresponding viral-induced malignancies in the mouse. The simultaneous detection test for reverse transcriptase and high molecular weight RNA was used to detect the presence of particles similar to RNA tumor viruses in human material. When this test was applied to human breast cancer, 79% of the malignant samples were positive for the simultaneous detection reaction and all of the control samples from normal and benign tissue were negative. Furthermore, the particles detected by this test localized in sucrose gradients at a density between 1.16 and 1.19 g/ml, the density characteristic of the oncogenic viruses. When the simultaneous detection test was applied to human leukemias, 22 of the 23 leukemic patients and none of the nonleukemic patients examined showed evidence of specific 70S RNA-directed DNA synthesis. To test the germ-line transmission of viral information (virogene hypothesis), the viral sequences contained in nonneoplastic DNA were removed by exhaustive hybridization with the viral probe to normal DNA in vast excess. The results indicate that the vast majority of normal cells do not contain a particular stretch of malignant information found in the cells of leukemic subjects. The same situation was found when cells from leukemic and nonleukemic identical twins were compared; the leukemic twin contained particle-related sequences which could not be detected in the WBC of the healthy sibling. The data do not support the virogene hypothesis, and are not consistent with the derepression of preexistent malignant sequences. The only etiologic mechanism which is supported is the one that requires the post-zygotic insertion of viral information into the genome.

2042 DEMONSTRATION OF ONCOGENIC POTENTIAL OF MAMMALIAN CELLS TRANSFORMED BY DNA-CONTAINING VIRUSES FOLLOWING PHOTODYNAMIC INACTIVATION. (Eng.) Li, J. L. H. (Milton S. Hershey Med. Cent., Pennsylvania State Univ., Hershey); Jerkofsky, M. A.; Rapp, F. *Int. J. Cancer* 15(2):190-202; 1975.

The oncogenic properties of hamster embryo cells transformed by herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) and simian virus 40 (SV40) were determined. Cell lines were grown from hamster embryo fibroblasts transformed by photodynamically-inactivated virus using neutral red dye. Newborn Syrian hamsters were inoculated sc with approximately 10^6 transformed cells from passages above 30. Of three HSV-1 clones, one produced palpable tumors within six weeks in 75% of the animals injected. All three HSV-2 clones (26, 32, and 42) were oncogenic. The HSV-2-26 and 32 lines produced a high rate of tumors (94% and 90%, respectively) within 2 and 4 wk, respectively. Only 21% of animals in the HSV-2-42 group produced tumors, and the latent period in this group was ten weeks. All animals injected with the 45-1 clones of SV40 developed tumors within two weeks. HSV-2 tumors showed fibrosarcoma, and metastases were seen in the lung in 70%. Of five hamsters that received sc inoculations of the HSV-2-32 primary tumor, four developed tumors within two weeks. Indirect immunofluorescence showed HSV-specific antigens in cytoplasm and/or on the surface of both the type 1 and 2 cultures. SV40 tumor antigen was detected in SV40 tumor cell nuclei. Sera from HSV or SV40 tumor-bearing animals were positive against HSV-infected hamster cells or SV40-infected monkey cells, respectively. The data support previous reports on the oncogenic properties of herpes simplex viruses. It is suggested that photodynamic therapy in the treatment of herpes infection may involve undesirable risks, including an increased cancer risk.

2043 AN ELECTRON MICROSCOPIC METHOD FOR STUDYING AND MAPPING THE REGION OF WEAK SEQUENCE HOMOLOGY BETWEEN SIMIAN VIRUS 40 AND POLYOMA DNAs. (Eng.) Ferguson, J. (Stanford Univ. Sch. Med., Calif.); Davis, R. W. *J. Mol. Biol.* 94(2):135-149; 1975.

A new procedure for investigating the possiblity of small amounts of partial DNA sequence homology between two defined DNA molecules was used to test for sequence homology between simian virus 40 (SV40) and polyoma DNAs. The procedure involves the construction of hybrid DNA molecules containing a SV40 DNA molecule covalently joined to a polyoma DNA molecule, using the sequential action of *Eco*RI restriction endonuclease and *Escherichia coli* DNA ligase. Denaturation of such hybrid DNA molecules then makes it possible to examine intramolecularly rather than intermolecularly renatured molecules. Visualization of these intramolecularly renatured "snapback" molecules with duplex regions of homology by electron microscopy revealed a 15% region of weak sequence homology. This region was denatured at about 35 C below the melting temperature of SV40 DNA and therefore corresponds to about 75% homology. This region was mapped on both the SV40 and polyoma genomes by the use of *Hemophilus parainfluenzae* II restriction endonuclease cleavage of the SV40 DNA prior to *Eco*RI cleavage and construction of the hybrid molecule. The 15% region of weak homology maps immediately to the left of the *Eco*RI restriction endonuclease cleavage site in the SV40 genome and halfway around from the *Eco*RI restriction endonuclease cleavage site in the polyoma genome. The results suggest that the region of homology does not code for the major capsid protein, but may code for a minor protein component of the virus particle.

2044 NON-HISTONE CHROMOSOMAL PROTEINS FROM VIRUS-TRANSFORMED AND UNTRANSFORMED 3T3 MOUSE FIBROBLASTS. (Eng.) Gonzalez, C. A. (Instituto de Medicina Experimental, U.C.V. Apartado 50587, Sabana Grande, Caracas, Venezuela); Rees, K. R. *Biochim. Biophys. Acta* 395(3):361-372; 1975.

The electrophoretic patterns and radioactive incorporation of nonhistone chromosomal proteins were compared in normal and Simian virus 40 (SV40)-transformed 3T3 mouse fibroblasts. Cells were labeled with either ^{14}C-protein hydrolysate, or ^{3}H-amino acid mixture at 1 mCi/l. ^{3}H-thymidine (0.2 mCi/l) was added to the cultures 30 min prior to harvesting. The nuclei were sepafated, and the chromatin was isolated by centrifugation. The chromosomal protein extract (containing 10 μg protein) was subjected to gel electrophoresis for 16 hr at 40 V, after addition of bromphenol blue. Twenty-two peaks were recognized in the absorbance profiles of the nonhistone proteins from both the log phase and density-inhibited normal cells, and the transformed cells. The density-inhibited cells gave a radioactive profile similar to that of the transformed cells. Peak 11 was a major peak in the normal log phase, but not the transformed cells. Transformed cells subcultured in media containing dibutryl cyclic AMP and theophylline showed a protein profile resembling that of the normal log phase cells. Density-inhibited cells stimulated to divide by addition of fresh medium showed profiles that varied throughout the cell cycle, but all fractions were continually present. Peak 11 showed a large increase with maximum absorbance at 10 hr (late G1) and a minimum at 30 hr. In synchronized cultures of transformed cells, however, peak 11 was evident throughout the cell cycle. It is concluded that there is no major qualitative alteration in the electrophoretic profile of nonhistone chromosomal proteins associated with the growth rate of 3T3 cells, but that there are quantitative differences, in particular the pronounced peak 11 in proliferating cells. The protein(s) of this peak are postulated to have a regulatory role in cell growth.

2045 STIMULATION OF NON-HISTONE CHROMOSOMAL PROTEIN SYNTHESIS IN SIMIAN VIRUS 40-INFECTED SIMIAN CELLS. Iida, H. (Inst. Med. Sci., Tokyo, Japan); Oda, K. *J. Virol.* 15(3):471-478; 1975.

Polyacrylamide gel electrophoresis was used to study nonhistone chromosomal proteins in simian virus 40

(SV40)-infected African green monkey kidney cells. Secondary monkey cell cultures in a resting state were infected with SV40, and synthesis of nonhistone chromosomal proteins was examined by labeling the cells with [^{3}H]leucine. Nonhistone proteins were fractionated by hydroxyapatite column chromatography. About 14 bands were seen on gel electrophoresis. An increase in the rate of synthesis was seen in almost all species of protein by 17 hr postinculation, when viral-induced synthesis of cellular DNA and messenger RNA (mRNA) was first observed. The rate of synthesis of nonhistone proteins continued to increase thereafter. Major peaks with molecular weights of about 48,000, 35,000 and 23,000 markedly increased after the onset of viral DNA replication (about 20 hr postinoculation). These peaks corresponded to the three major polypeptides of the SV40 virion. At 18 hr, incorporation of trichloroacetic acid-insoluble radioactivity was increased in infected cells, indicating the induction of cellular DNA synthesis. At 12-15 hr, radioactivity incorporated into residual acidic proteins increased about 1.3-fold; and the ratio of radioactivity incorporated into acidic proteins to that incorporated into basic proteins increased 1.7-fold, compared to values obtained at 0-3 hr. Nonhistone protein synthesis increased after five hours and reached a maximum at 10-15 hr when the replicative phase of infection began. Cells were infected with SV40 at either 20, 2, or 0.2 plaque-forming U/cell in the presence of 25 μg cytosine arabinoside/ml. Infection of cells at 2 and 20, but not at 0.2 plaque-forming U/cell resulted in a significant stimulation of nonhistone protein synthesis. These results suggest that stimulation of nonhistone protein synthesis is caused by an early SV40 function and that the induction of cellular DNA synthesis is not required for this stimulation.

2046 SIMIAN VIRUS 40 FUNCTIONS REQUIRED FOR THE ESTABLISHMENT AND MAINTENANCE OF MALIGNANT TRANSFORMATION. (Eng.) Martin, R. G. (Natl. Inst. Arthritis Metab. Dig. Dis., Bethesda, Md.); Chou, J. Y. *J. Virol.* 15(3):599-612; 1975.

The capacity of each of the five classes (A, B, BC, C and D) of simian virus 40 (SV40) mutants to produce characteristic alterations (transformations) in the growth of secondary Chinese hamster lung (CHL) cells was examined. The wild type SV40 was also used. Transformation assays were based on the capacity of CHL cells to escape contact inhibition of growth or to clone in low-serum medium. Class A mutants transformed cells at 33 C but not at 40 C by serial dilution; all other classes transformed at both temperatures. In all cases except the mock-infected controls, nearly confluent monolayers were seen when cultures were inoculated with 3 x 10^4 cells and incubated at 40 C. Only A mutants and mock-infected cultures had no dense clones. When 10^4 or fewer cells were plated, only A mutant and mock-infected cultures contained no clones. At 33 C all cultures but the mock-infected contained dense clones when the cultures were inoculated with 10^4 cells. Mock-infected confluent secondary CHL cells remained at low density in low serum at 40 C. Adsorption with wild type virions induced clones that overgrew the monolayer. Temperature-sensitive mutants of the B, C and BC complementation-derived groups transformed at 40 C. A mutants did not transform, and D mutants transformed poorly or not at all. Similar results were obtained when confluent monolayers of CHL cells were treated with virus and then incubated in depleted low-serum medium. Transformed clones were selected, purified by recloning and examined at both 33 C and 40 C. Of nine lines induced by the A mutants at 33 C, eight failed to maintain the transformed phenotype at 40 C, and virus was not recovered from the remaining line. These data indicate that A function is required for the maintenance of these growth characteristics transformation.

2047 FUNCTION OF SIMIAN VIRUS 40 GENE A IN TRANSFORMING INFECTION. (Eng.) Tegtmeyer, P. (Dep. Pharmacol. Microbiol., Case Western Reserve Univ., Cleveland, Ohio). *J. Virol.* 15(3):613-618; 1975.

The effect of simian virus 40 (SV40) temperature-sensitive gene A on the growth characteristics of transformed cells was studied with several well-characterized A mutants in a variety of host cells. Cellular growth was quantitated by simple colony assay. When Swiss/3T3 cells were first stably transformed by A28 or A30 at 33 C and then shifted to 39 C as dilute suspensions of single cells, the cells formed colonies with a transformed phenotype as did cells transformed by a wild type virus. Rabbit kidney cells were also tested (at 33 and 41.5 C) for temperature-sensitive characteristics of transformation by wild-type virus and SV40 mutants. The expression of the A gene appeared to affect the growth characteristics of rabbit cells stably transformed by A28 but not cells transformed by five other A mutants. The efficiency of colony formation by hamster embryo cells was studied after initiation of stable transformation at 33 C. Uninfected cells and cells transformed by wild type or A mutant virus were dispersed, serially diluted, and cultured at 33 or 40 C. The temperature shift decreased the capacity of the A-transformed cells to form colonies to the level of untransformed control cells. Fluorescent antibody assay for T antigen performed on each of the 3T3, rabbit, and hamster cell lines at either permissive or nonpermissive temperature showed that the antigen was present in each cell line. Since the outcome of the virus-cell interaction depended on the nature of the A mutation in a given cell species and on the species of the cell transformed by a given mutant, the transformation may require two distinct events, each related to A gene expression.

2048 SV40-TRANSFORMED CELLS WITH TEMPERATURE-DEPENDENT SERUM REQUIREMENTS. (Eng.) Toniolo, D. (New York Univ. Sch. Med., N.Y.); Basilico, C. *Cell* 4(3):255-262; 1975.

3T3 cells subconfluent at 32 C were infected with 5 plaque-forming units (PFU)/cell of simian virus 40 (SV40) that had been mutagenized by 20 μg/ml N-methyl-N'-nitro-nitrosoguanidine to isolate and char-

acterize a temperature-sensitive clone (ts23A). After 24 hr at 32 C in 10% serum, cells were diluted 20-fold, kept for 2-3 days in 10% serum at 32 C, incubated in 10% gammaglobulin-free serum, then incubated in 1% serum. After two days, cells were incubated at 39 C for two days; 5-fluorodeoxyuridine was then added at 30 μg/ml. Cells were washed and retreated twice, then transferred to 32 C. Ts23A cells were unable to form colonies in 1% serum at 39 C (less than 0.01% of plated cells yielded colonies), but were able to form colonies in 10% serum (3.5% yielded colonies). T-antigen, determined by fluorescent antibody staining, was present in ts23A and SV40-transformed 3T3 cells. Ts23A cells were able to form colonies on 3T3 monolayers, as were SV40-transformed 3T3 cells (ratio of colony yield per cell plated on 3T3/colony yield on plates: ts23A, 32 C, 0.96; 39 C, 1.26; SV40-transformed 3T3, 32 C, 0.87; 39 C, 1.18). Ts23A cells were unable to grow in agar suspension. As determined by ability to form plaques, virus rescued from ts23A cells was wild-type (ratio of PFU at 39 C/PFU at 32 C to wild-type SV40, 0.73; ts23A virus, 1.89). Unlike 3T3 cells, ts23A cells continued to synthesize DNA after 72 hr at 39 C in 1% serum (frequency of DNA-synthesizing cells as determined by autoradiography with ^{3}H-thymidine pulsing for one hour: 3T3 cells, 2.1%; ts23A cells, 13.9%). Ts23A cells had a 26% survival after two days in 1% serum at 39 C. Ts23A cells appear to represent transformed cell variants in which the ability to utilize or take up serum factors has become temperature-dependent.

2049 HUMAN BRAIN IN TISSUE CULTURE. III. PML-SV40-INDUCED TRANSFORMATION OF BRAIN CELLS AND ESTABLISHMENT OF PERMANENT LINES. (Eng.) Santoli, D. (Multiple Sclerosis Res. Center Wistar Inst., Philadelphia, Pa.); Wroblewska, Z.; Gilden, D. H.; Girardi, A.; Koprowski, H. *J. Comp. Neurol.* 161(3):317-328; 1975.

Human brain cells were transformed in culture by progressive multifocal leukoencephalopathy-Simian virus 40 (PML-SV40). Explants and monolayer cultures were taken from cases of multiple sclerosis, and monolayer cultures were also taken from cases of Jakob-Creutzfeldt disease, amyotrophic lateral sclerosis and noncentral nervous system disease. Explants were infected with 0.05 ml undiluted virus and monolayer cultures with over 30 plaque forming units/cell. Explants showed little or no cytopathic effects, but cell lysis was nearly always observed in monolayer cultures. Most cultures did not survive when infected during the stationary phase. The best results were obtained when they were treated with SV40 immune serum and infected during the proliferative phase. Transformation was indicated by the appearance of large numbers of small areas of cell overgrowth with higher density and higher incidence of mitotic figures than the surrounding cells. When cells entered the proliferative phase of transformation, passage at a split ratio of 1:4 or 1:6 resulted in the formation of monolayers within 2-3 days. Normal controls, passaged at a split ratio of 1:2, took 7-15 days to reach confluency. Cell density of transformed cultures was significantly greater than that of normal confluent cultures. A crisis phase similar to that in human diploid fibroblast transformation was seen in five of the monolayer cultures and one explant. Cell morphology remained unchanged after infection until the proliferative phase. Cells became rounded and epithelioid in appearance with a high nucleocytoplasmic ratio when confluency was reached. Various cultures were diploid, heteroploid and octoploid, and one had a combination of diploid and nearly tetraploid cells. All of the cells were positive for T-antigen. At the third transfer after infection, 6% of the cells were positive for V-antigen, but this dropped to zero by passage 14. SV40 immune serum treatment reduced the number of T-antigen positive cells. No infectious virus could be rescued from the culture medium of transformed cells, but repeated freezing and thawing of the cells resulted in isolation of infectious SV40. It is concluded that human brain cells can be transformed by PML-SV40 in a process roughly paralleling that of the transformation of human diploid fibroblasts.

2050 ISOLATION OF VARIANT CELLS FROM SV40-TRANSFORMED HUMAN DIPLOID FIBROBLASTS. (Eng.) Rosenberg, N. (Center for Cancer Res., Massachusetts Inst. of Technology, Cambrige, Mass.); Schaeffer, W. I.; Diamond, L. *Cancer Res.* 35(8):1970-1974; 1975.

Variant cell lines of human diploid fibroblasts (WI-38) that are sensitive to density-dependent inhibition of growth were isolated from three of four Simian virus 40 (SV40)-transformed human fibroblast cell lines. Variants were isolated by plating the transformed cells at low density, by treating them with 5-fluorodeoxyuridine or by growing them on glutaraldehyde-fixed monolayers of normal cells. The variant lines, isolated at a frequency of about 2% of all cells forming colonies after treatment, were initially recognized by colonial morphology, and the variant phenotypes were confirmed, after subculturing, by saturation-density determinations. The variant lines reached saturation densities plated 40% or less that those of the parent cell lines, and plated in soft agar medium at reduced efficiency. They retained SV40 T antigen. The modal chromosome numbers of two of the variant cell lines were increased, compared with those of the parent cell lines; two other variants were indistinguishable in chromosome number from the parent cells. Stability of these properties over a six month period was demonstrated with two of the variants. It is concluded that SV40-transformed human cell populations contain cells that do not express all of the properties associated with the transformed phenotype of the mass culture.

2051 B-CELL ORIGIN OF HAMSTER LYMPHOID TUMORS INDUCED BY SIMIAN VIRUS 40. (Eng.) Coe, J. E. (Rocky Mountain Lab., Natl. Inst. Allergy Infect. Dis., Hamilton, Mont.); Green, I. *Int. J. Cancer* 54(1):269-270; 1975.

The T- or B-cell origin of two lymphoid neoplasms induced by the oncogenic DNA simian virus 40 (SV40)

as studied. Hamster lymphoid neoplasm GD-36 (a ymphosarcoma composed of poorly differentiated lymphoblasts) and GD-248 (a lymphocytic leukemia that ecame a lymphoid tumor when transplanted) were xamined. Tumor cells were injected sc into Syrian amsters, and the tumors were harvested when they eached 3 cm in diameter (2-4 wk later). Almost all ells (80%-90%) of GD-36 and GD-248 tumors bound luorescein-labeled antibody to rabbit anti-hamster eagent (Fab) and 7Sγ_2-globulin, compared to 50% nd 5% for these characteristics in normal hamster ymphocytes. Tumor cells were uniformly negative hen tested with anti-7Sγ_2 (normal, 1%), γA (normal, %) and γM (normal, 25%) globulins. No rosettes ere formed when tested for C3 receptor by adding RBC coated with 19S rabbit antibody and mouse comlement, compared to about 30% of normal hamster splenic lymphocytes with both immunoglobulin and C3 receptor. The results indicate a B-cell origin for these tumors, probably derived from a 7Sγ_2 clone without the C3 receptor.

052 REQUIREMENT OF A CYTOPLASMIC FRACTION FOR SYNTHESIS OF SV40 DEOXYRIBONUCLEIC ACID N ISOLATED NUCLEI. (Eng.) DePamphilis, M. L. Stanford Univ. Med. Cent., Calif.); Berg, P. *J. iol. Chem.* 250(11):4348-4354; 1975.

n *in vitro* system in which lysate from simian virus 0 (SV40)-infected monkey cells can convert replicating SV40 DNA molecules to covalently closed, uperhelical DNA was further fractionated. About 0% of the SV40 DNA in the process of replication SV40(RI) DNA) completed replication in lysates of nfected BSC-1 cells by conversion to covalently losed, superhelical SV40 DNA (SV40(I) DNA). Fractionation of the lysate into nuclear and cytoplasic components blocked 99% of the synthesis of V40(I) DNA in the purified nuclei. Adding back he cytoplasmic fraction before incubation at 30 C ompletely restored the *in vitro* level of SV40(I) NA synthesis. Preliminary characterization of the ctivity found in the cytoplasmic fraction suggested t was a soluble, heat-labile protein(s) with a miniaum molecular wt of about 30,000 and an active sulfydryl group. The activity was present in both infected and uninfected monkey cells, and at a lower level in mouse, hamster, and human cell lines. Neither serum starvation nor cycloheximide treatment of cells diminished the activity in the cytoplasmic fraction. Purified cytoplasmic DNA polymerase from KB cells did not substitute for the cytoplasmic fraction which was required for elongation of newly synthesized DNA strands. In the absence of the cytoplasmic fraction, conversion of 4S DNA into longer strands was inhibited, and SV40(RI) DNA appeared to be broken specifically at the replication forks. An unfractionated cell lysate diluted 40-fold with proportional volume of assay mix and hypotonic buffer showed no decrease in SV40(I) synthesis. This indicates that although the soluble factor(s) required for conversion of SV40(RI) to SV40(I) DNA was found in the cytoplasmic fraction, the activity must be associated with the nucleus.

2053 CHARACTERIZATION OF T ANTIGEN IN CELLS INFECTED WITH A TEMPERATURE-SENSITIVE MUTANT OF SIMIAN VIRUS 40. (Eng.) Kuchino, T. (Inst. Med. Sci., Univ. Tokyo, Japan); Yamaguchi, N. *J. Virol.* 15(6):1302-1307; 1975.

T antigen induced in African green monkey kidney cells by a temperature-sensitive mutant of simian virus (*ts* 900), defective in a function required for cell transformation was characterized. The number of T antigen-positive cells estimated by an immunofluorescent technique was almost equal at permissive (32.5 C) and restrictive (38.5 C) temperatures, (the ratios of positive nuclei at 38.5 C to those at 32.5 C were 1.4 and 1.3 for the heat-labile and wild type viruses, respectively), but was slightly reduced when the infected cells were incubated at a higher temperature (40.5 C) (ratios 2.2 and 0.66, respectively). However, a complement fixation test indicated that the amount of T antigen induced by the mutant was not significantly different from that induced by the wild-type virus at 40.5 C. These results suggest that the T antigen-inducing ability of the mutant is not defective. Two distinct molecular species of T antigen were induced by the mutant at the permissive temperature, whereas only one form was observed at the restrictive temperature. The larger molecular form (14 to 15S) induced by the mutant at the permissive temperature was more heat labile than that induced by wild-type virus, suggesting that the mutated gene product may be T antigen or another component of the molecular form of the antigen.

2054 EXPRESSION OF SV40 T ANTIGEN DURING THE CELL CYCLE OF SV40-TRANSFORMED CELLS. (Eng.) Stenman, S. (Med. Nobel Inst., Karolinska Inst., Stockholm, Sweden); Zeuthen, J.; Ringertz, N. R. *Int. J. Cancer* 15(4):547-554; 1975.

The expression of the nuclear simian virus 40 (SV40)-induced T antigen was measured by microfluorimetry on individual, asynchronously growing SV40-transformed cells, which had been stained with hamster T antiserum. Pooled antiserum was obtained by transplanting into 6-wk-old Syrian hamsters 2×10^6 to 3×10^6 Syrian hamster HtSV40 cells. Serum from cancerous animals was diluted 1:8 in phosphate-buffered saline. A small area of fixed, unstained preparation was mapped with a *camera lucida*. Mapped C15s cells were measured with an aperture about 1/5 the width of a nuclear diameter. The product of the mean of three values and the smallest and largest nuclear diameter was used as a relative value for the amount of T antigen. The T antigen expression (F_{530}) and DNA content (E_{547}) were measured on the same cells by Feulgen microspectrophotometry. There was good linear correlation between F_{530} and E_{547}, with the best correlation obtained with the C15s line. The amount of T antigen was significantly higher in G2 cells than in G1 and S cells. The histograms for DNA and T antigen values measured in logarithmically growing cultures gave peaks, corresponding to G1 and G2 populations. In G1 cultures enriched by thymidine or cytosine arabinoside treatment, both DNA and T antigen clustered in regions corresponding to G1 in control cultures. Values of populations enriched by treatment with vinblastine sulfate were con-

centrated in the G2 region. T antigen expression increased during DNA replication and was twice as high in G2 as in G1 nuclei. Conversely, the T antigen expression was arrested at the G1 level in cells where DNA synthesis was blocked.

2055 FIBROBLAST SURFACE ANTIGEN PRODUCED BUT NOT RETAINED BY VIRUS-TRANSFORMED HUMAN CELLS. (Eng.) Vaheri, A. (Dept. Virology, Univ. Helsinki, SF-00290 Helsinki 29, Finland); Ruoslahti, E. *J. Exp. Med.* 142(2):530-535; 1975.

Human fibroblasts transformed with simian virus 40 (SV40), WI-38VAB and WI-SV40, were shown to lack cell type-specific surface (SF) antigen. SF antigen was demonstrated and localized by direct immunofluorescence with anti-SG-sheep IgG conjugated with fluorescein isothiocyanate and was purified from human plasma by cryoprecipitation, DEAE cellulose chromatography, and isoelectric focusing. Immunofluorescent staining revealed that SF antigen had a highly nonrandom fibrillar distribution on the surface of normal human fibroblasts. SV40-transformed cells had no detectable antigen. Radioactive anti-SF bound to normal cells, whereas binding to transformed cultures did not exceed the values obtained with SF antigen-negative cells. The SF content was at least ten times higher per milligram of cell protein than that in transformed cultures. SF antigen was found by immunodiffusion in concentrated culture fluid samples of normal fibroblasts and from transformed cells. Quantitation by radioimmunoassay showed that the culture fluids of SV40-transformed cells contained 30-40% of the SF antigen in fluids of normal cells. The results indicate that both normal and transformed cells produce SF antigen, but that the SV40 transformed cells do not retain the antigen on the surface.

2056 RNA OF SIMIAN SARCOMA-ASSOCIATED VIRUS TYPE 1 PRODUCED IN HUMAN TUMOR CELLS. (Eng.) Fidanián, H. M. (Univ. California Sch. Med., Los Angeles); Drohan, W. N.; Baluda, M. A. *J. Virol.* 15(3):449-457; 1975.

A simian-associated virus type 1 was studied as an experimental model for primate RNA viruses. The virus was purified from an established line of human rhabdomyosarcoma cells, W-20. Of five different methods for concentrating virus from cell culture supernatants, precipitation with 5% polyethylene glycol in the presence of 0.3 M NaCl consistently yielded the largest quantity of high-molecular weight viral RNA. This procedure primarily gave a single major precipitation band at 1.14 g/cm^3 after isopycnic sucrose sedimentation. Various amounts of a second minor peak at 1.21 g/cm^3 were occasionally seen. Virus was labeled with [5-^{3}H]uridine and [5-^{3}H]cytidine for six hours. When the ^{3}H-labeled viral RNA was extracted and analyzed by velocity sedimentation in 15-30% sucrose gradients, four major components were detected; they had sedimentation coefficients of 50-60S, 28-30S, 18-20S, and 4-10S. Thermal dissociation base composition analysis and polyadenylic acid content analysis were used to establish the origin of the different RNA species. Unlike the 50-60S RNA species, the smaller virion-associated RNAs lacked polyadenylic acid, and the 28-30S RNA had an average base composition similar to that of human ribosomal RNA. Upon heat denaturation, the native 50-60S RNA genome yielded polyadenylic acid-containing 28-30S subunits that degraded into 18-20S molecules upon further heat treatments. The 50-60S RNA had a guanine plus cytosine content of 56%. These data support the possibility that the simian sarcoma-associated virus type 1 virions produced in human cells are defective.

2057 RESPONSES OF INFANT RHESUS MONKEYS TO INOCULATION WITH MASON-PFIZER MONKEY VIRUS MATERIALS. *(Eng.)* Fine, D. L. (Natl. Cancer Inst., Frederick, Md.); Landon, J. C.; Pienta, R. J.; Kubicek, M. T.; Valerio, M. G.; Loeb, W. F.; Chopra, H. C. *J. Natl. Cancer Inst.* 54(3):651-658; 1975.

The clinical, pathologic and virus recovery studies related to *in vivo* responses to inoculation of Mason-Pfizer monkey virus (M-PMV) are reported. Six groups of a total of 68 newborn rhesus monkeys (*Mucaca mulatta*) were inoculated by either single or multiple routes, including s.c., i.p., i.v., i.m., intraocular, or intracerebral injections. Either M-PMV or M-PMV-infected cells were used. The clinical manifestations of the M-PMV inoculations were: generalized lymphadenopathy, development of nodules near inoculation site, significant weight loss, diarrhea, dehydration, vomiting, cyanosis, rashes, and central nervous system impairment. The highest mortality (80%) occurred when inoculations were given at birth; 52% mortality occurred after inoculation at one day after birth and 41% mortality occurred when inoculated later than two days. Most deaths were attributed to viral or bacterial pneumonia. Other conditions were: involuted thymus, pancreatitis, hemosiderosis, nephritis, and brain edema. There were no great hematologic differences although there were several cases of basophilia, anemia and neutropenia. Three abnormal patterns in serum protein were found: total hypoproteinemia, hyperbeta globulinemia and hypergamma globulinemia but these did not correlate with inoculum, clinical course or lesions. Virus particles characteristic of M-PMV were confirmed by electron microscopy in bone marrow, lymph nodes and kidneys. Two virus particles were found: intracytoplasmic type A, and an extracellular form. The viral influence on the thymus implies that M-PMV exerts an immunosuppressive effect through the thymus. The results also indicate that M-PMV exhibits symptoms common to other oncornaviruses.

2058 PROPERTIES OF VISNA VIRUS PARTICLES HARVESTED AT SHORT TIME INTERVALS: RNA CONTENT, INFECTIVITY, AND ULTRASTRUCTURE. *(Eng.)* Brahic, M. (Departement de Biologie Moleculaire, Faculte de Medecine, Secteur Nord, 13326 Marseille Cedex 3, France); Vigne, R. *J. Virol.* 15(5):1222-1230; 1975.

he RNA content, infectivity and ultrastructure of isna virus particles and their similarity to other umor viruses was studied. Radioactively labeled isna virus was harvested every five min (early harested). After purification, RNA was extracted rom the suspension. ^{3}H-labeled RNA was also preared from Visna virus harvested after 18 hr labelng. Both RNA preparations were subjected to elecrophoresis in mixed polyacrylamide-agarose gels, ither native or heat denatured. ^{32}P-labeled Moloney train of murine sarcoma leukemia virus [M-MVS (MLV)] as added before loading on the gels, to serve as nternal marker. Visna viruses from an 18 hr harest gave a single peak of RNA of lower mobility han M-MSV(MLV) RNA and corresponded to 60-70S RNA. fter melting this RNA it was converted to a 30-40S pecies. Early harvested Visna virus separated into wo peaks of native RNA: a 60-70S and a new series hat showed an electrophoretic mobility higher than -MSV(MLV) RNA. Similar amounts of radioactive reovery between harvest times indicated that there as no change in the rate of virus release from the ells. This early harvest RNA sedimentation coeficient was identical to that of 30-40S RNA subnit obtained by heat denaturation. In 1.8% acrylaide gels, without agarose, the mobilization of his early 30-40S subunit showed a lower value than hat of heat-denatured 60-70S RNA. However, the wo RNAs comigrated exactly when the free subunits resent in early virus were heat-denatured prior to lectrophoresis. These early harvested subunits ere found to be precursors of the 60-70S RNA but here was a significant difference in infectivity. n electron microscopic study, the early harvest NA was of the dense core type with few clear centers. The rate of maturation of RNA, in this case, s slow but does not influence infectability.

059 VIRAL GENESIS OF HUMAN TUMORS. (Ger.) Grossgebauer, K. (Inst. f. Hygiene und ed. Mikrobiologie, 1000 Berlin 65, Fohrer Str. 4, East Germany). *Therapiewoche* 25(17):2350, 352, 2355-2358; 1975.

060 CONCATEMERIC INTERMEDIATES IN ADENO-VIRUS-ASSOCIATED VIRUS DNA REPLICATION abstract]. (Eng.) Sebring, E. D. (Natl. Inst. ealth, Bethesda, Md.); Straus, S. E.; Ginsberg, . S.; Rose, J. A. *Fed. Proc.* 34(3):639; 1975.

061 A STUDY OF REPRODUCTION CONDITIONS OF THE AVIAN MYELOBLASTOSIS VIRUS FOR THE ECOVERY OF REVERSE TRANSCRIPTASE ENZYME. (Rus.) raevskaia, N. A. (Inst. Poliomyelitis Viral ncephalitides, U.S.S.R. Acad. Med. Sci., Moscow, .S.S.R.); Sito, A. F.; Dumina, A. L. *Biull. ksp. Biol. Med.* 79(6):56-59; 1975.

062 TRANSCRIPTION OF NATURAL AND ARTIFICIAL mRNAs BY RNA-DIRECTED DNA POLYMERASE abstract]. (Eng.) Weiss, G. B. (Molecular Hemaology Branch, NHLI, NIH, Bethesda, Md. 20014); alvey, A. K.; Kantor, J. A. *Fed. Proc.* 34(3):531; 975.

2063 DETECTION AND CHARACTERIZATION OF RNA TUMOR VIRUS SPECIFIC NUCLEOTIDE SEQUENCES IN AVIAN CELL DNA [abstract]. (Eng.) Evans, R. M. (Univ. California, Los Angeles). *Diss. Abstr. Int. B* 35(11):5543-5544; 1975.

2064 PILOT EXPERIMENTS WITH EB VIRUS IN OWL MONKEYS (*AOTUS TRIVIRGATUS*). III. SEROLOGICAL AND BIOCHEMICAL FINDINGS IN AN ANIMAL WITH RETICULOPROLIFERATIVE DISEASE. (Eng.) Epstein, M. A. (Univ. of Bristol Medical Sch., Univ. Walk, Bristol BS8 1TD, England); zur Hausen, H.; Ball, G.; Rabin, H. *Int. J. Cancer* 15(1):17-22; 1975.

2065 INFLUENCE OF THE VIRUS OF NUCLEAR POLYHEDROSE OF *GALLERIA MELLONELLA* AND ITS DNA ON THE CHROMOSOMES OF MAMMALIAN CELLS. (Ukr.) Buzhievs'ka, T. I. (Inst. Molecular Biology and Genetics, Acad. Sciences, Ukrainian S.S.R.); Nikonenko, V. U.; Vavilina, I. V. *Dopov. Akad. Nauk RSR Ser. B* (3):249-252; 1975.

2066 ROLE OF PITUITARY IN LEUKEMOGENESIS [abstract]. (Eng.) Bentley, H. P., Jr. (Univ. South Alabama, Mobile); Hughes, E. R.; Peterson, R. D. A. *Fed. Proc.* 34(3):833; 1975.

2067 LATENT INFECTION OF SENSORY GANGLIA WITH HERPES SIMPLEX VIRUS: EFFICACY OF IMMUNIZATION. (Eng.) Price, R. W. (Natl. Inst. Dent. Res., Bethesda, Md.); Walz, M. A.; Wohlenberg, C.; Notkins, A. L. *Science* 188(4191):938-940; 1975.

2068 INHIBITION OF HERPES SIMPLEX VIRUS REPLICATION BY araT. (Eng.) Gentry, G. A. (Sch. Med., Univ. Mississippi, Jackson); Aswell, J. F. *Virology* 65(1):294-296; 1975.

2069 CHARACTERIZATION OF THE CLOSED CIRCULAR, DOUBLE-STRANDED SUPERCOILED FORM OF MOLONEY LEUKEMIA PROVIRUS DNA [abstract]. (Eng.) Weinberg, R. A. (Cent. Cancer Res., Massachusetts Inst. Technol., Cambridge); Gianni, A. M.; Smotkin, D.; Rozenblatt, S. *Fed. Proc.* 34(3):609; 1975.

2070 PROSTAGLANDIN LEVELS IN VIRUS AND CHEMICALLY-INDUCED MOUSE TUMORS [abstract]. (Eng.) Pelus, L. M. (Rutgers, State Univ., Newark, N.J.); Humes, J. L.; Strausser, H. R. *Fed. Proc.* 34(3):828; 1975.

2071 CLONAL CELL LINES FROM A FERAL MOUSE EMBRYO WHICH LACK HOST-RANGE RESTRICTIONS FOR MURINE LEUKEMIA VIRUSES. (Eng.) Hartley, J. W. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.); Rowe, W. P. *Virology* 65(1):128-134; 1975.

2072 SUPPRESSION OF MURINE VIRUS LEUKAEMOGENESIS BY THIOGLYCOLLATE, A BACTERIOLOGICAL CULTURE MEDIUM THAT AFFECTS MACROPHAGE PEROXIDASE. (Eng.) Strauss, R. R. (Albert Einstein Med. Cent., Philadelphia, Pa.); Friedman, H.; Mills, L.; Zayon, G. *Nature* 255(5506):343-344; 1975.

2073 HEXOKINASE ISOENZYME VARIATIONS AND SUBCELLULAR DISTRIBUTION OF HEXOKINASE ACTIVITY IN UNINFECTED AND MURINE SARCOMA VIRUS-TRANSFORMED BALB 3T3 CULTURES [abstract]. (Eng.) Lakshmi, M. V. (St. Louis Univ. Sch. Med., Mo.); Kumari, H. L., Bose, S. K. *Fed. Proc.* 34(3):576; 1975.

2074 SPECIFIC PHOSPHOPROTEINS ARE STRUCTURAL COMPONENTS OF MAMMALIAN ONCORNAVIRUSES [abstract]. (Eng.) Pal, B. K. (Univ. South. California Sch. Med., Los Angeles); Roy-Burman, P. *Fed. Proc.* 34(3):637; 1975.

2075 DENATURATION OF POLYOMA DNA BY PHAGE T4 GENE 32 PROTEIN. (Eng.) Monjardino, J. (R. Free Hosp. Sch. Med., London, England); James, A. W. *Nature* 255(5505):249-252; 1975.

2076 POLYOMA VIRUS STRAIN WITH ENHANCED SYNTHESIS OF CAPSID PROTEIN. (Eng.) Tachovsky, T. G. (Wistar Inst. Anatomy and Biology, Philadelphia, Pa. 19104); Hare, J. D. *J. Virol.* 16(1):116-122; 1975.

2077 PURIFICATION AND PROPERTIES OF DNA POLYMERASE FROM RAUSCHER LEUKEMIA VIRUS [abstract]. (Eng.) Bandyopadhyay, A. K. (Natl. Cancer Inst., Frederick, Md.). *Fed. Proc.* 34(3):531; 1975.

2078 TWO FORMS OF VIRAL DNA POLYMERASES (R-MULV) WITH DIFFERENT BIOCHEMICAL AND IMMUNOLOGICAL PROPERTIES [abstract]. (Eng.) Wu, A. M. (Bionetics Res. Lab., Bethesda, Md.); Cetta, A.; Sarngadharan, M. G.; Gallo, R. C. *Fed. Proc.* 34(3):531; 1975.

2079 MORPHOLOGICAL AND CYTOCHEMICAL CHARACTERISTICS OF THE HUMAN CELLS TURNED MALIGNANT BY THE ROUS SARCOMA VIRUS AND POLYOMA VIRUS. (Rus.) Karazhas, N. V. (Dept. Etiology and Immunodiagnostics Tumors, Inst. Epidemiology and Microbiology Acad. Medical Sci. U.S.S.R., Moscow, U.S.S.R.); Shevliagin, V. Ia.; Amchenkova, A. M. *Tsitologiia* 17(7):829-834; 1975.

2080 PLASMINOGEN-INDEPENDENT FIBRINOLYSIS BY PROTEASES PRODUCED BY TRANSFORMED CHICK EMBRYO FIBROBLASTS [abstract]. (Eng.) Chen, L. B. (Massachusetts Inst. Technol., Cambridge); Buchanan, J. M. *Fed. Proc.* 34(3):532; 1975.

2081 3' TERMINAL POSITION OF SV40 NUCLEOTIDE SEQUENCES IN mRNA FROM SV40 TRANSFORMED CELLS [abstract]. (Eng.) Ghosh, P. K. (Yale Med. Sch., New Haven, Conn.); Sotman, S.; Lebowitz, P. *Fed. Proc.* 34(3):674; 1975.

2082 PARAMETERS INFLUENCING T-ANTIGEN EXPRESSION AND TRANSFORMATION IN SV40 VIRUS-INFECTED HUMAN CELLS [abstract]. (Eng.) Kaplan, M. M. (Meloy Lab., Springfield, Va.); Blattner, W. A.; Mason, T.; Lubiniecki, A. S.; Fraumeni, J. F. *Fed. Proc.* 34(3):834; 1975.

2083 SURFACE PROPERTIES OF CLONED SPONTANEOUSLY TRANSFORMED MOUSE CELL LINES AFTER SV40 INFECTION [abstract]. (Eng.) Pancake, S. J. (Natl. Inst. Health, Bethesda, Md.); Chang, C.; Luborsky, S.; Couvillion, L.; Mora, P. T. *Fed. Proc.* 34(3):850; 1975.

2084 INTERACTIONS OF FIBROBLASTS WITH INSOLUBLE ANALOGUES OF CELL SURFACE CARBOHYDRATES [abstract]. (Eng.) Chipowsky, S. (Johns Hopkins Univ., Baltimore, Md.); Schnarr, R.; Roseman, S. *Fed. Proc.* 34(3):614; 1975.

2085 HIGH MOLECULAR WEIGHT PROTEINS IN SPONTANEOUSLY AND IN SV40 TRANSFORMED MOUSE CELLS [abstract]. (Eng.) Coll, J. (Natl. Inst. Health, Bethesda, Md.); Couvillion, L.; Mora, P. T. *Fed. Proc.* 34(3):614; 1975.

2086 REPLICATION OF LACTIC DEHYDROGENASE VIRUS AND SINDBIS VIRUS IN MOUSE PERITONEAL MACROPHAGES. INDUCTION OF INTERFERON AND PHENOTYPIC MIXING. (Eng.) Lagwinska, E. (Washington Univ. Sch. Med., St. Louis, Mo.); Stewart, C. C.; Adles, C.; Schlesinger, S. *Virology* 65(1):204-214; 1975.

See also:

* (Rev): 1804, 1806, 1807, 1841, 1842, 1847
* (Chem): 1909
* (Immun): 2097, 2102, 2109, 2110, 2111, 2121, 2122, 2140, 2141, 2142, 2146, 2158, 2172, 2186, 2189
* (Epid): 2305
* (Path): 2211, 2231, 2256, 2291

2087 THE ROLE OF THE SPLEEN AND IMMUNITY IN THE METASTATIC PATTERN OF A MURINE RETICULUM CELL SARCOMA. (Eng.) Faraci, R. P. (Natl. Cancer Inst., Bethesda, Md.); Schour, L. *J. Surg. Oncol.* 7(1):85-92; 1975.

The effect of additional splenic tissues on the behavior of a reticulum cell sarcoma (RCS), which metastasizes selectively to the spleen in C3H/HeN mice, was investigated. The behavior of this tumor following transplantation into immunosuppressed mice was also investigated. In two series of experiments, the incidence of nonsplenic visceral metastases in 12 pairs of normal mice, parabiosed to normal partners injected sc with RCS cells (1×10^6), was compared with that in 11 normal mice injected with RCS cells; 14 splenectomized, tumor-injected mice; 13 sham-operated, tumor-injected mice; and nine pairs of normal, RCS-treated mice parabiosed with splenectomized mice. Fewer than 20% of the animals with two spleens (parabiotic group) developed gross liver metastases compared with 93% of the splenectomized mice and 50% of the mice with one spleen (control, sham-operated, and parabiotic splenectomized groups). The splenectomized group also had the highest incidence (64%) of kidney metastases and the parabiotic group had the lowest incidence (8%). The same trend was encountered when the incidence of microscopic metastases in the lungs and kidneys of tumor-injected animals was examined; the incidence was highest in the animals with no splenic tissue and lowest in the group with two spleens. The incidence of visceral metastatic spread in animals with one spleen was again intermediate between the parabiotic and splenectomized groups. In a third series of experiments, 28 C3H received 550 R wholebody irradiation followed by im injection of RCS cells (10^6) from C57/B16J mice; the animals were sacrificed on day 14 or day 21. Controls received tumor-cell injections but were not irradiated. There was no difference in the incidence of gross or microscopic metastases in the spleen, liver, kidneys, and lungs between the controls and the immunosuppressed animals, either 14 or 21 days after tumor-cell injection. Thus, the protective effect of additional spleen against visceral metastases of RCS is not immunologic.

2088 SYSTEMIC PROTECTION AGAINST RADIATION. I. EFFECT OF AN ELEMENTAL DIET ON HEMATOPOIETIC AND IMMUNOLOGIC SYSTEMS IN THE RAT. (Eng.) Pageau, R. (Department of Nuclear Medicine, Centre Hospitalier Universitaire, Université de Sherbrooke, Sherbrooke, Québec, Canada); Lallier, R.; Bounous, G. *Radiat. Res.* 62(2):357-363; 1975.

The effects of normal laboratory pellet food and the Mead-Johnson elemental diet 3200-BD on the survival of Sprague-Dawley rats given a total body dose of 700 rads of ^{60}Co γ-radiation were determined. The diets were also compared with respect to their effects on [^{3}H]-thymidine incorporation by hematopoietic tissues, on activation of the immune system by SRBC (10^8 cells, ip), and on serum proteins. Only 6% of 16 rats fed the food pellets survived wholebody exposure to 700 rads and the mean survival time was nine days. In contrast, 73% of 15 rats fed the elemental diet for a week before irradiation survived and the mean survival time was 59 days. However, none of 15 animals given the elemental diet after radiation survived, and the mean survival time of 6.1 days was significantly shorter than that obtained with the normal diet. In animals fed the normal diet, [^{3}H]-thymidine incorporation was highest in the mesenteric lymph nodes followed by the spleen, thymus, and bone marrow. This same sequence was obtained in animals on the elemental diet; however, [^{3}H]-thymidine incorporation was significantly higher in spleen (162%), thymus (181%), and bone marrow (164%) of these rats compared with the pellet-fed controls. Antibody titers seven days after activation of the immunologic system by SRBC were 179 in animals given the elemental diet and 89 in those given the pellets. The elemental diet also produced a significant increase in the serum α_2 fraction. These results confirm the reported radioprotective action of the Mead-Johnson diet and demonstrate that feeding of the diet is associated with enhanced cellular proliferation in hematopoietic tissues and better response to antigen stimulation.

2089 THE PROCOAGULANT FACTOR OF LEUKAEMIC PROMYELOCYTES: DEMONSTRATION OF IMMUNOLOGIC CROSS REACTIVITY WITH HUMAN BRAIN TISSUE FACTOR. (Eng.) Gouault-Heilmann, M. (Service Central d'Hématologie-Immunologie, Hopital Henri Mondor, Creteil, France); Chardon, E.; Sultan, C.; Josso, F. *Br. J. Haematol.* 30(2):151-158; 1975.

The procoagulant activity of leukemic promyelocytes was examined using immunological techniques. The promyelocyte extract (PME) was obtained by disruption of leukemic promyelocytes, centrifugation, lyophilization of the supernatant, and final reconstitution of the lyophilized product in distilled water. The protein component of tissue factor was prepared from a human brain acetone powder by freeing the powder of lipid with heptane-butanol, solubilization of the apoprotein by extraction with 0.25% sodium deoxycholate, and chromatography on Sepharose 6 B. The lipid component of the brain powder was extracted with chloroform. Tissue factor activity of the different preparations was determined in a one-stage system involving 0.1 ml test plasma, 0.1 ml test material, and 0.1 ml 0.025 M $CaCl_2$. The test plasma was either normal plasma or plasma from patients with factor VII deficiency. Although the protein complement of tissue factor was devoid of significant procoagulant activity and phospholipids at high concentration exhibited an inhibitory activity, a mixture of the two had a potent activity. This was demonstrated by shortening of the clotting time of normal or hemophilic plasma, contrasting with the absence of any significant effect on factor-VII deficient plasma. Promyelocyte extract exhibited a clot promoting activity of the same type as that of the tissue factor. Antisera to human brain tissue factor and to crude PME were produced in rabbits by footpad injection of the antigen mixed with complete Freund's adjuvant. The anti-brain tissue factor antisera neutralized the tissue factor activity of the promyelocyte extract, and antisera against the promyelocytes neutralized the activity of the human

brain extract. In Ouchterlony tests, a reaction of partial identity appeared between one component of the promyelocytes extract and one component of the brain tissue factor. The data thus demonstrate that the promyelocyte procoagulant is antigenically related to brain tissue factor.

2090 QUANTITATIVE STUDY OF THE EFFECT OF PREVIOUS *TRICHINELLA SPIRALIS* INFECTION ON SARCOMA 180 ASCITIC TUMOR FORMATION IN MICE. (Eng.) Lubiniecki, A. S. (Meloy Lab., Inc., 6715 Electronic Drive, Springfield, Va. 22151); Cypess, R. H. *Tropenmed. Parasitol.* 26(3):329-334; 1975.

A study was carried out to determine if infection with *Trichinella spiralis* influenced the development of a transmissible ascitic tumor (Crocker Sarcoma 180, S-180). A new system in which sarcoma development was easily and nondestructively quantitated by linear regression analysis on the basis of ascites production (increased mass) was devised for this purpose. This procedure readily separated the postinoculation period into incubation and clinical phases. The former was dependent on S-180 dose, while the latter was not. *T. spiralis* infection 28 days prior to S-180 challenge produced small but statistically significant increases in the length of both the incubation period and survival time in HaM/ICR rats that had been inoculated ip with S-180, but did not affect the clinical phase. Administration of tumor cells 56 days following the helminthic infection had no detectable effect on any parameter of tumor development studied. The results are consistent with the hypothesis that *T. spiralis* infection temporarily altered the host reaction to S-180, possibly at the level of non-specific macrophage activity.

2091 *H-2*-LINKED GENETIC CONTROL OF RESISTANCE TO HISTOCOMPATIBLE TUMORS. (Eng.) Williams, R. M. (Harvard Med. Sch., Boston, Mass.); Dorf, M. E.; Benacerraf, B. *Cancer Res.* 35(6):1586-1590; 1975.

The role of the major histocompatibility complex of the mouse (H-2) in resistance to two transplanted histocompatible tumors was evaluated by determining the differences in survival times between the syngeneic parent strain and various F_1 hybrids. C57BL-/10nSn (B10) mice and their F_1 hybrids were given injections of a methylcholanthrene-induced fibrosarcoma of B10 origin (5,000 cells, sc). The B10 X B10.BR F_1, B10 X B10.M F_1, B10 X B10.WB F_1, and B10 X 5R F_1 hybrids survived significantly longer than the B10 parental strain or B10 X B10.D2 F_1 and B10 X 18R F_1 animals, while B10 X 2R F_1 mice succumbed significantly sooner than any of the above groups. Statistical comparisons of geometric mean survival times of the strain of tumor origin (B10) *versus* the F_1 hybrids showed the influence of genes coded for the H-2 complex in the phenomenon, termed "hybrid resistance" or "allogeneic inhibition". However, tumor resistance did not occur in all hybrids and could not be attributed to a single dominant Ir gene localized in the I region. In a second group of experiments, the mean survival times of DBA/2 x B10.D2 F_1 animals given injections of the P815 mastocytoma of DBA/2 (D2) origin was compared to the mean survival times of various hybrids with the D2 parent. Again,the results demonstrated the importance of the H-2 gene complex in this phenomenon. Simple I-region control of tumor resistance *via* immune response genes cannot adequately explain the results obtained with all tumors.

2092 LYMPHOCYTE ANTIBODY INTERACTION IN CYTOTOXICITY AGAINST HUMAN TRANSITIONAL CELL CARCINOMA. (Eng.) Hakala, T. R. (Veterans Adm. Hosp., Minneapolis, Minn.); Lange, P.H.; Castro, A. E.; Elliott, A. Y.; Fraley, E. E. *J. Urol.* 113(5):663-667; 1975.

The interaction of antibodies and lymphocytes and their immune reaction against human transitional cell carcinoma (TCC) was studied by an *in vitro* microcytotoxicity assay. Target cell lines were derived from: (1) TCC of the renal pelvis, ureter, and bladder, (2) renal cell carcinomas, (3) normal bladder epithelium, kidney, and testis (obtained from patients at surgery), and (4) human embryonic skin, muscle, and kidney cell strains. The lymphocytes of TCC patients more frequently displayed significant cytotoxicity against TCC target cells (83%) than did those of control patients (31%). There was no significant difference between the percentage of TCC and control donors with lymphocytes cytotoxic to target cells derived from renal cell carcinoma or normal kidney or testis. A non-complement dependent IgG antibody was detected in the serum of occasional TCC patients which induced cytotoxicity against TCC target cells by lymphocytes from donors with and without TCC. Lymphocytes from TCC donors were more sensitive to activation by anti-TCC lymphocyte dependent antibody suggesting that the surface of lymphocytes from some TCC donors is coated *in vivo* with an anti-TCC lymphocyte dependent antibody; this may be a significant factor in immunity to TCC of the urinary tract.

2093 COMPARATIVE *IN VITRO* SENSITIVITY OF TWO METHYLCHOLANTHRENE-INDUCED MURINE SARCOMA LINES TO HUMORAL AND CELLULAR IMMUNE CYTOTOXICITY. (Eng.) Bataillon, G. (Fondation Curie - Institut du Radium, Bat. 110, 91405 Orsay, France); Pross, H.; Klein, G. *Int. J. Cancer* 16(2):255-265; 1975.

The sensitivity of MC57M and G, derivatives of two methylcholanthrene-induced murine C57Bl sarcomas to immune cytotoxic, cytostatic or cytolytic spleen cells and sera was compared in parallel *in vitro* assays. In order to discriminate between cytotoxicity and cytostasis (which were both measured by a microcytotoxicity test and a cloning-inhibition test), target cells were labeled either before (cytotoxicity test) or after (cytostasis test) being incubated with the immune effectors. The level of cross-reactivity displayed by the two lines was found to depend on the nature of the immune effector rather than on the assay which was used. It was high with immune spleen lymphocytes, alone or in the presence of decomplemented antisera, and low

with antisera in the presence of rabbit complement. MC57G cells were more sensitive than MC57M cells to both effectors. Both cell lines were insensitive to antibody-dependent cell-mediated cytotoxicity. Preliminary evidence is presented suggesting a probable involvement of embryonic and Moloney leukemia virus-induced cell surface antigens in the *in vitro* sensitization of the two tumor lines to immune sera.

2094 CELL-MEDIATED CYTOTOXIC REACTIONS TO TUMOR ASSOCIATED ANTIGENS. *(Eng.)* Herberman, R. B. (Natl. Cancer Inst., Bethesda, Md.). *J. Reticuloendothel. Soc.* 17(4):236-240; 1975.

A review of recent data concerning cell-mediated cytotoxic reactions towards antigens associated with tumors is presented. Natural cell-mediated cytotoxicity of cells from rats and mice appears to be against antigens associated with endogenous C-type viruses. Many adult humans have been found to have cytotoxic reactivity against human leukemic cells, and some human sarcoma cell lines. There is also evidence that immune reactivity is T-cell mediated when directed against allogeneic target cells, and that a variety of cell types are active against syngeneic tumor cells. The author suggests that the complexity of findings may be due to 1) the different assay systems used, 2) the ability of many different cells to become cytotoxic *in vitro*, 3) the presence of multiple cell surface antigens on the same cell and 4) the presence of natural immunity in healthy populations. The author proposes that natural human cytotoxic reactivity may be due to sensitization to antigens associated with viruses that are ubiquitous and possibly oncogenic.

2095 STUDIES ON THE PHYSIOLOGICAL MANIFESTATIONS OF CELL MEDIATED CYTOTOXICITY. II. INHIBITION OF [^{3}H]THYMIDINE INCORPORATION BY PLASMACYTOMA CELLS EXPOSED *IN VITRO* TO SENSITIZED SPLENOCYTES. (Eng.) Steinitz, M. (Hebrew Univ.-Hadassah Medical Sch., Jerusalem, Israel); Feigis, M.; Weiss, D. W. *Cell. Immunol.* 17(1):181-191; 1975.

The early metabolic changes effected in target cells under cellular immune attack were measured by tritiated thymidine uptake to explain the mechanism of cell mediated immunity (CMI) and to develop reliable techniques for its assessment. Splenocytes (effector cells) were obtained from normal allogeneic C57Bl and syngeneic BALB/c male mice and from animals of both strains which had received an i p inoculation of living plasmocytoma (PCT) cells (3×10^7 cells and 1×10^6 cells, resp.). PCT was derived from a tumor induced in a BALB/c male by repeated i p injection of mineral oil. Incorporation of tritiated thymidine by PTC cells *in vitro* was markedly and rapidly inhibited after one to several hours by contact with splenocytes from allogeneic and syngeneic donors inoculated with the neoplastic cells. Inhibition was dependent on the presence of intact effector cells in direct contact with the targets; the degree of inhibition increased with elevation in the ratio of effector to target cells. Irradiation of the allogeneic splenocytes reduced or abolished their inhibitory capacity. Supernatants of interactions between PCT and sensitized allogeneic spleen cells had no specific inhibitory activity. Effector cells from allogeneic mice sensitized with PCT were capable of target cell thymidine uptake inhibition and of ^{51}Cr liberation from labeled target cells. Syngeneic effectors, slightly higher in thymidine uptake inhibition, could not bring about ^{51}Cr liberation. Thus, the degree of effector sensitization, or the nature of the antigens against which sensitization is effected, may determine the manifestations of CMI. It is suggested that inhibition of DNA precursor incorporation may offer a rapid and accurate means of assessing cytostatic effects mounted by sensitized effector cells.

2096 AUGMENTED ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY FOLLOWING SENSITIZATION OR NON-SPECIFIC STIMULATION OF HUMAN EFFECTOR CELLS. (Eng.) Connolly, J. M. (Natl. Cancer Inst., Building 10, Room 4B17, Bethesda, Md. 20014); Schwartz, R. H.; Handwerger, B. S.; Wunderlich, J. R. *Transplantation* 20(3):186-193; 1975.

Human peripheral blood leukocytes were stimulated *in vitro* with mitogens (poke-weed mitogen, concanavalin A, and phytohemagglutinin) or allogeneic cells. Each form of stimulation augmented the cytotoxic effector cell activity of lymphoid cells in a 4-hr test for antibody-dependent cell-mediated cytotoxicity. This augmented activity did not involve release of detectable nonspecific toxins, nor did it require the presence of mitogen during the cytotoxicity test. Stimulated attacking cells appeared more cytotoxic either because of a more potent cytotoxic mechanism per individual cytotoxic cell or because of an increased percentage of cytotoxic cells. The finding that leukocyte stimulation augments effector cell function *in vitro* suggests that stimulated host cells at a graft site may be more effective than nonstimulated cells in the antibody-dependent cell-mediated cytotoxic pathway for graft destruction.

2097 IMMUNODEPRESSION BY ROWSON-PARR VIRUS IN MICE: EFFECT OF ROWSON-PARR VIRUS AND FRIEND LEUKEMIA COMPLEX INFECTIONS ON CONTACT SENSITIVITY IN SUSCEPTIBLE AND RESISTANT MICE. (Eng.) Bendinelli, M. (Inst. Microbiol., Univ. Pisa, Italy); Campa, M.; Toniolo, A. *Infect. Immun.* 11(5):1031-1037; 1975.

Contact sensitivity to 2-phenyl-4-ethoxymethilene oxazolone as a probe for cell-mediated immunity, was investigated in susceptible female inbred BALB/c and resistant outbred C57BL/6 mice after infection with Friend leukemia complex (FLC) or Rowson-Parr virus (RPV). Generally, 0.2 ml of a 2% ethanol solution of the drug was applied to about 4 cm^2 of the abdominal wall. Six days after sensitization, the ears of the mice were painted with 1% oxazolone, which induced ear swelling in the sensitized mice. In BALB/c mice, FLC depressed contact sensitivity when given before primary sensitization but had no effect on established contact sensitivity nor on the response elicited by a booster application of

the sensitizer. These findings, together with the failure to alter reactivity to an aspecific inflammatory stimulus, indicate that FLC impairs the afferent limb of the response. In the same strain of mice RPV infection did not significantly depress contact sensitivity, as judged by the extent of the reaction 24 hr after challenge, but slightly inhibited the early antibody-mediated phase of this reaction. In C57BL/6 mice, neither viral preparation affected contact sensitivity. Dissociation between development of leukemia and depression of antibody response has been previously observed following FLC virus infection; this is apparently not the case in the cell-mediated immunity in this study.

2098 IMPAIRED CELL-MEDIATED IMMUNITY IN PATIENTS WITH CANCER. (Eng.) Buda, J. A. (Francis Delafield Hosp., 99 Fort Washington Ave., New York, N.Y. 10032); Suciu-Foca, N.; Blomain, E.; McManus, S.; Reemtsma, K. *J. Surg. Oncol.* 7(6):525-529; 1975.

The ability of peripheral blood lymphocytes to respond *in vitro* to phytohemagglutinin (PHA) and to allogeneic cells in mixed leukocyte reaction (MLC) was studied in 85 patients with cancer (gastrointestinal tract, 32; breast, 53 cases) and in 50 healthy controls. The effect produced by sera from cancer patients on *in vitro* lymphocyte blastogenesis was tested on autologous cells and on homologous cells from a constant panel of ten normal volunteers. The MLC response of lymphocytes from patients with localized cancer was 13,417 cpm; from patients with metastatic cancer, 7,510 cpm; and from the normal volunteers, 33,753 cpm; in the presence of autologous serum. Similar effects were obtained with homologous serum. The MLC response of lymphocytes from the volunteers was inhibited 23% by sera from patients with localized cancer; 51% by sera from those with regional cancer; and 58% by sera from those with metastatic cancer. Normal serum gave 6% inhibition. Similar but smaller effects on PHA response were found.

2099 IMMUNE MECHANISMS IN LEUKEMIA: SUPPRESSION OF CELLULAR IMMUNITY BY STARVATION. (Eng.) Martinez, D. (Univ. Michigan Medical Sch., Ann Arbor, Mich. 48104); Cox, S.; Lukasewycz, O. A.; Murphy*, W. H. *J. Natl. Cancer Inst.* 55(4):935-939; 1975.

A model for studying the effect of starvation on cell-mediated immunity to malignant lymphocytes (syngeneic lymphoid cells) is described. C58/Wm mice were starved 1-3 days before or after immunization. The capacity of starved animals to survive immunization was used to quantify immunosuppression. When starvation bracketed immunization by -1 to +1 days, only 2 of 23 mice survived primary immunization, compared with 100% survival for nonstarved controls. A two-day period of starvation +1 to +7 days after primary immunization reduced survival about 30%. Mice were immunized, starved, and then challenged with viable l_b cells. When mice were starved from -3 to +1 days before or after challenge, there was a 25-45% decrease in survival. Starvation caused a disproportionate depletion of lymphoid tissue elements. The proportional loss in the weight of the spleen and thymus was essentially twice as great as the loss in total body weight. The peripheral blood WBC count was reduced by about 20% when mice were starved one day and by approximately 50% when they were starved two days. When mice were starved 1-2 days, the differential WBC count did not shift and there was no significant change in the number of blood RBC or in the hematocrit. Starvation for two days caused a 65-70% reduction in the number of viable mononuclear spleen cells. Starvation for three days caused about 90% reduction. Adoptive cell transfer experiments showed that the immunocompetence of individual spleen immunocytes was not reduced by starvation.

2100 ISOLATION OF AN IMMUNOSUPPRESSIVE PEPTIDE FRACTION FROM THE SERUM OF CANCER PATIENTS. (Eng.) Nimberg, R. B. (Boston Univ. Med. Cent., Boston, Mass.); Glasgow, A. H.; Menzoian, J. O.; Constantian, M. B.; Cooperband, S. R.; Mannick, J. A.; Schmid, K. *Cancer Res.* 35(6):1489-1494; 1975.

An investigation was carried out to define the immunological hyporeactivity of the sera of 53 patients with solid tissue cancer, and to distinguish this from the sera of normal volunteers. When the sera of the cancer patients were subjected to gel filtration, membrane partition, and ion-exchange chromatography, an active immunosuppressive peptide fraction was isolated from the sera of 27 patients. The fraction was heterogeneous as judged by high-voltage electrophoresis, and suppressed both the phytohemagglutinin-induced proliferation of lymphocytes *in vitro* and the *in vivo* induction of splenic plaque-forming cells in CD-1 mice. The specific activity of this peptide fraction was significantly increased over that of the unfractionated starting material. Protein fractions that were suppressive at 1-6 mg/ml prior to ultrafiltration were significantly suppressive at 0.1-0.3 mg/ml after filtration. No active peptide fraction could be obtained from normal controls (five individual sera and a pool of 12 individuals) or in a group of 28 noncancer-bearing hospitalized individuals. Although α-globulin-containing DEAE peaks isolated from the sera of the cancer patients were also immunosuppressive, the major amount of immunosuppressive activity was present in a fraction that appeared to be devoid of α-globulins. It is possible that the active factor is initially carried by an α-globulin, but, as carrier sites become saturated, is picked up by proteins that chromatograph in non-α-globulin fractions.

2101 CHARACTERIZATION OF A CHIMPANZEE ANTI-HUMAN MELANOMA ANTISERUM. (Eng.) Stuhlmiller, G. M. (Duke Univ. Medical Center, Durham, N.C., 27710); Seigler, H. F. *Cancer Res.* 35(8): 2132-2137; 1975.

The production of an anti-melanoma antiserum in a chimpanzee by hyperimmunization with human melanoma cells was facilitated by the similarities of serum proteins, A and O blood group antigens, and HL-A antigens in the chimpanzee and man. Melanoma cells were obtained from a patient who had A- blood, and typed HL-A 7, 9, 10. A female chimpanzee typed the

ame. Complete Freund's adjuvant was required for he third booster inoculation in order to induce roduction of antibody cytotoxic to cultured melaoma cells. Target cells used for complementlependent cytotoxicity testing of immune serum ncluded normal human peripheral lymphocytes, ultured melanoma cell lines, normal skin fiproblasts from HL-A phenotyped donors, skin fibroblasts from melanoma patients, fetal fibroblasts, continuous lines of HeLa, Hep, and KB cells, and nonmelanoma neoplastic cell lines (including an osteosarcoma, a meningioma, a neuroblastoma, and carcinomas of the lung and stomach). The IgG fraction of the immune serum was prepared by DEAE-Sephadex A-50 chromatography. After absorption of the antiserum with peripheral blood lymphocytes of the tumor donor, it was specifically cytotoxic to melanoma cells from 14 cell lines and to cells from eight fetal fibroblast lines. Such absorbed antiserum was negative when tested against a large panel of normal peripheral blood lymphocytes and fibroblasts, and against eight nonmelanoma cell lines. Subsequent absorption with melanoma cells from any of seven sources removed all antimelanoma and all antifetal reactivity. Similar absorption with fetal cells removed all antifetal cytotoxicity, but did not remove all of the antimelanoma cytotoxicity. The IgG fraction of the antiserum possessed the cytotoxic activity. The data demonstrate the presence of at least two distinct tumor-associated antigens on cell membranes of melanoma cells, one being shared by melanoma cells and the other by fetal fibroblasts.

2102 PASSIVE IMMUNISATION OF MARMOSET MONKEYS AGAINST NEOPLASIA INDUCED BY A HERPESVIRUS. (Eng.) Laufs, R. (Hygiene Institut der Universitat, D-34 Gottingen, Kreuzbergring 57, West Germany); Steinke, H. *Nature* 255(5505):226-228; 1975.

The prevention of herpesvirus-induced malignant lymphomas by passive immunization was studied in cotton topped (CT) marmoset monkeys (*Saguinus oedipus*). One of three sera used for passive immunization was obtained from a CT marmoset that had received 2 x 10^6 freshly prepared tumor cells from a marmoset with herpesvirus saimiri (HVS)-induced malignant lymphomas. The neutralization index of this serum was 3.0. A monkey immunized im with 6 ml of the serum before iv challenge with a 10^{-1} dilution of live HVS [median tissue culture infective dose ($TCID_{50}$) = 1,000] showed delayed tumor development and survived 134 days. The nonimmunized control died of malignant lymphoma 33 days after HVS inoculation. The second serum was obtained from four CT marmosets inoculated with 10 $TCID_{50}$ of cell-free HVS. The neutralization index of the pooled sera from these monkeys was 2.8. While three nonimmunized control monkeys died of malignant lymphoma 23-45 days after inoculation of a 10^{-3} dilution of HVS ($TCID_{50}$ = 10), two of threee monkeys immunized with 6 ml of serum died of the tumor as late as 73 and 79 days, and the third survived without any sign of clinical disease or HVS infection. The third serum was obtained from CT marmosets actively immunized with a killed HVS vaccine. The neutralization index of the serum pool was 3.5 and the titer of complement-fixing antibodies was 1:32. Two monkeys immunized with 6 ml of this serum died 63 and 74 days after challenge with a 10^{-1} virus dilution, whereas the nonimmunized controls died 28 and 34 days after inoculation. All of four passively immunized monkeys that received a 10^{-3} dilution were alive 204 days after inoculation. In contrast, all of the nonimmunized controls died 23-46 days after receiving the 10^{-3} dilution. That protection could be transmitted with the serum of vaccinated monkeys indicates that humoral antibodies were important in the observed resistance to HVS. The results of passive immunization also support the concept that HVS is the etiological agent for malignant lymphomas.

2103 COMPARATIVE STUDY OF MEMBRANE AND INTRACYTOPLASMIC IMMUNOGLOBULIN CLASSES IN HUMAN LYMPHOID CELLS. (Eng.) Ferrarini, M. (Inst. of Industrial Medicine, Univ. of Genoa, Genoa, Italy); Bargellesi, A.; Corte, G.; Viale, G.; Pernis, B. *Ann. N.Y. Acad. Sci.* 254:243-253; 1975.

The hypothesis that the appearance of IgM molecules in lymphocytes bearing IgD represents a differentiation step for lymphocytes before they transform into secreting plasma cells was tested. Because of their monoclonal nature, chronic lymphocytic leukemia (CLL) cells appeared useful for studying the expression of IgD and IgM molecules in a homogenous cell population. Tonsils, on the other hand, served as a source of lymphoid cells at all stages of maturation. Before use, the different lymphocyte preparations were freed of phagocytic cells by a carbonyl iron technique. Fluorescence methods were used for detecting membrane immunoglobulin classes. Starting with cells which had been labeled by incorporation of ^{14}C-labeled amino acids, immunoglobulins present in cell secretions (supernatants of centrifuged cultures) and in cell cytoplasm (supernatants of centrifuged, Nonidet 40-lysed cells) were detected by polyacrylamide gel electrophoresis of SDS-dispersed trichloracetic acid precipitates. Classes of Ig present in secretions or cytoplasm were identified and quantitated by immunoprecipitation with rabbit antisera against purified IgG, IgA, IgM, and IgD immunoglobulins, purified κ and λ chains, and $F(ab)_2$ fragment, again starting with labeled cells and ending with measurement of radioactivity of the precipitates. Results obtained for membrane immunoglobulin on CLL lymphocytes showed that IgD was present on a vast percentage of lymphocytes in the majority of cases studied and that, between extreme cases of cells positive for IgM and IgD only, all intermediate variants could be detected. This implied that the ratio of IgD and IgM molecules varied in different cells and suggested that a maturation process occurred in the CLL clones. Results of tests on secretions and cytoplasm showed that although there was no significant production of immunoglobulin molecules in four cases, in two others there was active IgM synthesis. Similarly, results of experiments with lymphoid cells from tonsils showed that a considerable fraction of these cells bore membrane immunoglobulin and also secreted immunoglobulin. It is concluded that the findings are compatible with the hypothesis that the switchover from IgD to IgM production is a clonal maturation phenomenon.

2104 HUMAN MYELOMA IgG HALF-MOLECULES: STRUCTURAL AND ANTIGENIC ANALYSES. *(Eng.)* Spiegelberg, H. L. (Scripps Clin. Res. Found., La Jolla, Calif.); Heath, V. C.; Lang, J. E. *Biochemistry* 14(10):2157-2163; 1975.

A structural and antigenic analysis of a human κ, IgG myeloma protein isolated from plasma and urine samples of a patient before and after chemotherapy was performed to determine the structural abnormality that resulted in the presence of this half-molecule. Following purification of the IgG fraction, aliquots were subjected to papain and neuraminidase digestion, cyanogen bromide cleavage, and purification of residues on Sephadex G-50 columns. Amino acid analysis and peptide mapping were performed, as were starch gel and polyacrylamide gel electrophoresis of the whole and half IgG molecules. Antigenic analysis was performed using antisera against normal human IgG and antisera produced against the myeloma half-molecule. The results indicate that the myeloma protein had a heavy chain of smaller molecular wt than that of normal γ chains, but that the amino acid composition of the peptides containing the residues responsible for inter-chain bonding were similar in the normal and myeloma proteins. No Fc fragment of the myeloma protein could be prepared, due to its extreme susceptability to papain. The myeloma protein was found to be antigenically deficient in the Fc fragment and did not precipitate with anti-Fc antisera. The 7S protein from the myeloma patient could be dissociated into half-molecules, and both the 7S and half-molecules had similar genetic markers on the first and second homology region of the γ chain. The half-molecule did not have the genetic markers on the third homology region. The results suggest that a deletion in the third homology region of the myeloma protein is the structural abnormality which results in the lack of noncovalent interaction in the Fc fragment.

2105 A HUMAN LYMPHOID CELL LINE SECRETING IMMUNOGLOBULIN G AND RETAINING IMMUNOGLOBULIN M IN THE PLASMA MEMBRANE. (Eng.) Prekumar, E. (Univ. California Los Angeles Sch. Med.); Singer, P. A.; Williamson, A. R. *Cell* 5(1):87-92; 1975.

A selected clone, LA 85.2, of a human lymphoid cell line was studied for production of different classes of immunoglobulins and types of chains. The biosynthesis of immunoglobulins was followed by observing the incorporation of radioactivity during three hours of incubation with ^{35}S-methionine. The labeled immunoglobulins were characterized by precipitation with specific antisera, followed by dissolution of the precipitates with sodium dodecyl sulfate (SDS) and analysis by polyacrylamide gel electrophoresis in the presence of SDS. The only labeled immunoglobulin secreted was a 7S molecule, which, upon reduction and alkylation, was shown to contain ν chains and L chains. The intracellular immunoglobulins found after lysis of the cells showed, in addition to the ν and L chains, a third peak running in an intermediate position between the ν and L chains. This peak, having a molecular wt of about 36,000 daltons, does not correspond to any known immunoglobulin polypeptide chain. Biosynthesis of immunoglobulins during labeling with ^{35}S-methionine for 24 hr revealed the synthesis of μ chains (in addition to ν chains, L chains and the novel 36,000 dalton component). Additional experiments using 24 hr labeling revealed that an assembly of μ chains and light chains produced an 8S IgM that was retained in the plasma membrane. The IgM was produced at a slow rate and in lesser amounts that the IgG. Based on unpublished observations showing that the novel 36,000 dalton polypeptide coprecipitates with other antigen-antibody complexes, it is hypothesized that this substance has the property of binding to some common feature on immunoglobulin molecules. This substance may play a role in holding the surface IgM in the plasma membrane.

2106 SERUM I-K (IMMUNOCONGLUTININS) IN HODGKIN'S DISEASE. (Eng.) Merucci, P. (Regina Elena Inst. Cancer Res., Rome, Italy); Abbolito, M. R.; Barduagni, A. *Biomedicine* 23(2):52-54; 1975.

The presence of increased C'3 fraction observed in Hodgkin's disease was confirmed and the behavior of C'3 fragments in lymphoproliferative diseases was investigated. Serum was collected by venipuncture from 26 normal adults and 33 patients at various clinical stages of Hodgkin's disease. Mean C'3 concentration was consistently higher in patients than in controls (189.53 mg : 100 ml ± 20.47 mg : 100 ml *versus* 134.25 mg : 100 ml ± 23.75 mg : 100 ml). Immunoelectrophoresis of the sera of Hodgkin's disease showed a precipitation band with an anti-human serum in the IgM region. Conglutinins present in patients with Hodgkin's disease may be considered as immunoconglutinins (I-K).

2107 MEMBRANE-BOUND IMMUNOGLOBULINS ON HUMAN LEUKEMIC CELLS. EVIDENCE FOR HUMORAL IMMUNE RESPONSE OF PATIENTS TO LEUKEMIA-ASSOCIATED ANTIGENS. (Eng.) Metzgar, R. S. (Duke Univ. Med. Cent., Durham, N.C.); Mohanakumar, T.; Miller, D. S. *J. Clin. Invest.* 56(2):331-338; 1975.

The detection of immunoglobulins on the membranes of human leukemic cells by a microcytotoxicity technique is described. A significant percentage of lymphocytes from normal donors failed to react with goat antisera to human heavy chain determinants or to λ-light chains. Lymphocytes from some normal donors, however, did react with antisera to κ-light chains. A high percentage (50-90) of cells from some leukemic patients were killed by antisera to light chains and by one or more antisera to heavy chain determinants. Trypsin treatment of leukemic cells resulted in a loss of cytotoxic activity with all immunoglobulin antisera. Reactivity with the κ-light chain antiserum was detectable two hr after trypsinization of chronic myeloid leukemic (CML) cells and eight hr after treatment of acute lymphocytic leukemic (ALL) cells. Reactivity with the antisera to heavy chain determinants and λ-light chains could not be detected 8 and 48 hr after trypsinization of CML and ALL cells, resp. The cytotoxic activity of the immunoglobulin antisera to heavy chains was abolished by absorption with the

pecific immunoglobulin used to define the antisera y precipitation. Eluates (pH 3.2) prepared from eukemic cells which reacted by cytotoxicity with he immunoglobulin antisera were shown to contain mmunoglobulins of different heavy chain classes. n addition, some of the eluates had cytotoxic ntibody activity to human leukemia cells. The pecificity of the eluted antibodies is similar to he specificity previously described for cytophilic ntibodies from leukemic patients and nonhuman priate antisera to human leukemia cells. The possible n *vitro* detection and *in vivo* significance of the luted non-complement-fixing antibodies is consiered.

108 ANTIBODY STIMULATION OF TUMOUR GROWTH IN T-CELL DEPLETED MICE. (Eng.) Fink, M. . (Washington Univ. Sch. Med., St. Louis, Mo.); arker, C. W.; Shearer, W. T. *Nature* 255(5507): 04-405; 1975.

he effects of anti-L cell serum (aLS) on tumor rowth *in vivo* were studied in thymectomized C3H/He ice. The mice were subjected to γ-radiation (850 ads) and reconstituted with syngeneic bone marrow ells 5 x 10^6 to 10 x 10^6, iv) one day before inoulation with L-929 cells (2 x 10^6, sc). The antierum was administered ip in doses of 5, 50, or 500 l on the day preceding tumor inoculation; in one xperiment, additional 50 μl doses were given on ays 5, 10, 15, and 20 after tumor inoculation. umor growth in mice receiving single or multiple njections of 50 μl aLS increased significantly on ays 9, 19, and 25. A 10-fold increase or decrease n dose abrogated the immunostimulating effect of LS. Preliminary experiments with congenitally thymic nude mice confirm the low level of host anitumor immunity observed in thymectomized, irradated, and bone marrow-reconstituted C3H/He mice. t is therefore improbable that enhancement of tuor growth was caused by a blocking effect of aLS n tumor allograft rejection. The key event *in vivo* s apparently an alteration in tumor-cell physiology ccurring as a direct consequence of interaction ith antibody.

109 ANTIBODY PRODUCTION AND INTERACTION WITH LYMPHOID CELLS IN RELATION TO TUMOR IMMUITY IN THE MOLONEY SARCOMA VIRUS SYSTEM. (Eng.) arada, M. (Shionogi Res. Lab., Osaka, Japan); Pearon, G.; Redmon, L.; Winters, E.; Kasuga, S. *J. mmunol.* 114(4):1318-1322; 1975.

he membrane immunofluorescence (MF) and the antiody-dependent normal lymphocyte cytotoxicity (ADNLC) ssays were used to follow the development of antiodies to Moloney sarcoma virus (MSV)-induced antiens with respect to tumor growth in male $CD2F_1$ mice. MF antibody titers rose to high levels between two and four weeks following MSV inoculation in mice, which developed tumors that subsequently regressed. Antibody was seen 14 days post-inoculation in all mice, but remained at low levels in mice which developed progressively growing tumors. ADNLC was not detected in the sera of mice with progressively growing tumors, but was detected about six weeks after virus inoculation in sera of mice with regressing tumors. Sera from mice immunized to the MSB cell line (an MSV-induced tumor) enhanced the cytotoxicity of normal spleen cells and of spleen cells from mice inoculated with 10^4 immune spleen cells. This enhancement was reduced if 10^6 cells were injected. An inverse relationship between the activation of these cells in the ADNLC assay and the development of cellular immunity was noted. The results indicate that the rise in MF titers in regressor mice corresponds to the initiation of tumor regression.

2110 ANTIBODIES TO EPSTEIN-BARR-VIRUS: ANTIGENS BEFORE AND AFTER THE DEVELOPMENT OF BURKITT'S LYMPHOMA IN A PATIENT TREATED FOR HODGKIN'S DISEASE. (Eng.) Magrath, I. (Makerere Univ., Kampala, Uganda); Henle, W.; Owor, R.; Olweny, C. *N. Engl. J. Med.* 292 (12):621-623; 1975.

The case history of an eight-year-old Ugandan boy who developed Burkitt's lymphoma subsequent to treatment for Hodgkin's disease is presented. Epstein-Barr virus (EBV)-associated antigens were seen well before Burkitt's lymphoma (BL) was detected. The EBV-associated antigens rose gradually before the diagnosis of BL, and then rose faster. Antibodies to the diffuse and restricted components were initially absent, but rose suddenly with the onset of BL. High titers persisted, despite therapy, until death. The authors suggest that Burkitt's lymphoma may arise in virus carriers long after the primary infection, due to the action of some factor(s) that affects EBV-infected lymphoid cells preferentially.

2111 AUGMENTATION OF LYMPHOCYTE CYTOTOXICITY BY ANTIBODY TO HERPESVIRUS SAIMIRI ASSOCIATED ANTIGENS. (Eng.) Prevost, J. M. (Natl. Cancer Inst., Bethesda, Md.); Orr, T. W.; Pearson, G. R. *Proc. Natl. Acad. Sci. USA* 72(5):1671-1675; 1975.

Experiments were undertaken to determine the possible mechanism by which antibodies directed against Herpesvirus saimiri (HVS)-associated cell membrane antigens could participate in immunity to cells infected or transformed by HVS. The antibody-dependent lymphocyte cytotoxicity (ADLC) of rhesus monkey lymphocytes against HVS-infected owl monkey kidney cells was assayed in the presence of two different dilutions (1:60 and 1:240) of normal and an anti-HVS positive serum. ADLC activity of the lymphocytes was markedly enhanced by incubation with serum containing HVS antibodies. At a lymphoid to target cell ratio of 400:1, the activity induced by both serum dilutions was approximately 75%. Although ADLC activity decreased with decreasing numbers of lymphocytes, it was significantly higher than control values at every ratio tested including 20:1. ADLC titers tended to increase with increasing membrane immunofluorescence (MF) titers. However, with some sera, the ADLC titers were much higher than those determined by MF, suggesting that multiple serum factors were involved in mediating ADLC. Absorption of both low- and high-titer serum with HVS-infected owl monkey kidney cells removed all ADLC activity.

Thus, if ADLC is mediated by multiple factors in higher-titer sera, the factors are all directed against HVS-associated antigens expressed in productively infected cells. Preliminary results from serial serum samples from two infected monkeys that developed leukemia and/or lymphoma demonstrated a progressive increase in ADLC but not MF titers with progression of disease. This finding again suggests the participation of multiple humoral factors in the mediation of ADLC activity. It is suggested that this mediation may involve an antibody species different from the antibody directed in the MF test, but directed at the same antigen; multiple antibodies directed against a variety of HVS-associated membrane antigens; or antigen-antibody complexes.

2112 ROSETTING OF ANTIBODY-SENSITIZED TUMOR CELLS WITH ANTIIMMUNOGLOBULIN ANTIBODY-COATED ERYTHROCYTES BY A NEW METHOD FOR DETECTING ANTIGENS ON CELLS. (Eng.) Molinaro, G. A. (Univ. of Illinois at the Medical Center, Chicago, Ill. 60680); Sato, K.; Paque, R. E.; Dray, S. *Cancer Res.* 35(8):2213-2216; 1975.

A new method for detecting antigens on cells was developed. Tumor cells are treated with rabbit antibody to tumor-associated cell surface antigens and tested with SRBC coated with antibody specific for the sensitizing rabbit immunoglobulin. Under these conditions, the sensitized tumor cells form rosettes with the RBC. The tumor cell lines used were line 1 and line 10 ascitic variants of diethylnitrosamine-induced hepatomas of strain 2 guinea pigs. The antisera were obtained by iv injection of hepatoma cells into rabbits and absorption with spleen and liver cells from normal guinea pigs. The anti-line 10 antiserum was also adsorbed with line 1 cells. The RBC were coated with purified anti-b4 or anti-b5 anti-Ig antibodies. By this method, it was demonstrated that line 1 and line 10 hepatoma cells bear antigens not present on normal liver cells and also that the two lines bear antigenically different tumor-associated antigens. This rosetting procedure is considered to be simpler than that of the mixed anti-globulin reaction and other serological methods such as C1 fixation, immunofluorescence, or cytotoxicity. The method may be a general one for detecting and enumerating cells bearing surface antigens.

2113 EXPRESSION BY HUMAN NEUROBLASTOMA CELLS OF AN ANTIGEN RECOGNIZED BY NATURALLY OCCURRING MOUSE ANTI-BRAIN AUTOANTIBODY. (Eng.) Martin, S. E. (Natl. Heart and Lung Inst., NIH, Bethesda, Md. 20014); Martin, W. J. *Cancer Res.* 35(9):2609-2612; 1975.

A number of human tumor cell lines of both neural and nonneural origin were assayed for the expression of an interspecies brain antigen (mouse brain antigen 2), detected by naturally occurring antibodies in normal mouse sera. Aliquots of serum from normal C3Hf/HeN mice were absorbed with either human neuroblastoma or nonneural cell lines. They were tested for residual cytotoxic activity against NB1 cells derived from a neuroblastoma adrenal metastasis of a primary ovarian teratoma of a C3H/HeIcrf mouse. Mouse brain antigen 2 was present on four human neuroblastoma cell lines but not on other human lines tested, including four glial cell lines and a retinoblastoma. This antigen could be readily detected on normal brain and kidney tissue of both mice and man and is therefore a normal tissue, rather than a tumor-specific, antigen. Normal mouse serum, absorbed with the neuroblastoma and glial cell lines, was tested for cytotoxic activity against murine N-T16 neuroblastoma cells and murine teratoma-derived Ter-A cells; both cell lines lacked mouse brain antigen 2. These findings demonstrate the value of naturally occurring antibodies in normal sera for the detection and serological classification of human tumor antigens and indicate that caution is required in attempts to distinguish tumor-specific and tissue-specific antigen expression on human neuroblastoma cells.

2114 A STUDY OF CYST FLUID AND PLASMA CARCINOEMBRYONIC ANTIGEN IN PATIENTS WITH CYSTIC OVARIAN NEOPLASMS. (Eng.) van Nagell, J. R., Jr. (Univ. Kentucky Med. Cent., Lexington); Pletsch, Q. A.; Goldenberg, D. M. *Cancer Res.* 35(6):1433-1437; 1975.

Cyst fluid and plasma carcinoembryonic antigen (CEA) levels were measured in 11 patients with ovarian cystadenocarcinoma and in 16 patients with benign ovarian neoplasms. In patients with ovarian cancer, plasma CEA levels were not elevated above 2.5 ng/ml unless cyst fluid CEA levels were 4-16 μg/ml. Cystic and plasma CEA levels were elevated most consistently with mucinous ovarian tumors. On the basis of molecular size and immunoreactivity by immunodiffusion, ovarian cancer cyst fluid CEA and colonic cancer CEA had similar immunochemical properties. Consistent with the findings in other neoplasms, follow-up studies showed that plasma CEA levels returned to the normal range 2-12 wk after excision of the tumor. Since plasma CEA is apparently more consistently increased in patients with mucinous cystadenocarcinoma, the CEA blood assay may be of value in the management of these patients.

2115 CARCINOEMBRYONIC ANTIGEN-IDENTIFICATION OF A TRI-N-ACETYLCHITOTRIOSYL TYPE STRUCTURE AS AN IMMUNODETERMINANT GROUP. (Eng.) Anderson, B. (Northwestern Univ. Medical Sch., Chicago, Ill., 60611); Jameson, A.; Hirata, A. A.; Safford, J. W.; Tomita, J. T. *Immunochemistry* 12(6/7):577-580; 1975.

In an effort to identify immunodeterminant groups on the carcinoembryonic antigen (CEA) molecule, a number of mono- and oligosaccharides were utilized as potential inhibitors in a radioimmunoassay. The CEA was prepared by perchloric acid extraction of primary colon carcinoma tissues followed by Sepharose and Sephadex chromatography and radioiodination. Anti-CEA was prepared in guinea-pigs. The radioimmunoassay was a solid-phase procedure in which guinea-pig anti-CEA γ-globulin was coated on polystyrene tubes, followed by the addition of inhibitor and, finally, of ^{125}I-CEA. The abilities

f various inhibitors to compete with ^{125}I-CEA for nti-CEA combining sites were expressed as the perent of total ^{125}I-CEA displaced by the particular nhibitor added. Tri-N-acetylchitotriose, a triaccharide of β1→4-linked N-acetylglucosamine, reulted in up to 50% inhibition of binding, whereas i-N-acetylchitobiose, N-acetylglucosamine and its -anomer were less efficient as inhibitors. The ri-N-acetylchitotriose had ten times the inhibiory activity of the di-N-acetylchitobiose, and at east 100 times the activity of N-acetylglucosamine. he specificity of inhibition by tri- and di-N-aceylchitobiose was supported by the inactivity or light activity of nine other mono- and oligosacchaides. The studies indicated, therefore, that at east a portion of the anti-CEA antibodies had a ombining site with an affinity for the trisacchaide unit of two N-acetylglucosamine residues in 1→4 linkage, as well as another N-acetylglucosamine oiety or possibly the second β-anomeric linkage of ri-N-acetylchitotriose.

116 PRELIMINARY IMMUNOLOGIC CHARACTERIZATION OF GLYCOPROTEIN ANTIGENS (CEA) OF TUMORS F GASTROINTESTINAL TRACT IN HUMANS. (Eng.) Harożińska, A. (ul. Marcinkowskiego 1, 50-368 Wrolaw, Poland); Zawadzka, H.; Woźniewski, A.; Richer, R. *Arch. Immunol. Ther. Exp. (Warsz.)* 23(2): 47-255; 1975.

pecific antigens of human primary carcinomas of the ectum colon and stomach were studied. Specimens of ucous membrane surrounding such tumors were used as ontrol material to exclude the problem of alloantienic differences between donors of tumors and noral tissues. Normal gastrointestinal mucosa, liver, pleen, and kidney served as material for absorption nd for additional control studies. Extraction of ntigens from tumor and normal tissues was performed sing perchloric acid. Immune sera were prepared in abbits injected sc with solutions of lyophilized xtracts and Freund's complete adjuvant. For absorpion of the immune sera, appropriate doses of lyohilized normal organs or human plasma were deterined by a precipitation inhibition test. All imunologic tests were made by double diffusion in aar. Immunoelectrophoretic studies were performed n electrophoretically separated extracts of the noral and tumor tissues, using unabsorbed and absorbed abbit antitumor immune sera. Antigens from carcinmas of all areas had an electrophoretic mobility of -globulin and gave lines of identity in the double iffusion test. This antigen was not demonstrable n the mucous membranes surrounding the carcinomas, n normal organs, or in gastrointestinal tissues of uman fetuses. Further tests indicated its lack of elation to human serum orosomucoid, ceruloplasmin, aptoglobin, transferrin, α_2-macroglobulin and α-etoprotein. It was not determined whether the anigen in this study was the counterpart of the carinoembryonic antigen of Gold.

117 HL-A ANTIGEN FREQUENCIES IN MYELOMA. (Eng.) Mason, D. Y. (Radcliffe Infirmary, oodstock Road, Oxford OX2 6HE, England); Cullen, P. *issue Antigens* 5(4):238-245; 1975.

The frequency of HL-A antigens was determined in 63 myeloma patients and in 83 normal subjects. The patients consisted of 32 males and 31 females, with an average age of 60. The distribution of paraprotein types was IgG: 37, IgA: 15, light chain disease: 10, and IgD: 1. The ages of the controls ranged from 18-64. Tissue typing was performed using a microlymphocytotoxicity test with lymphocytes separated from defibrinated venous blood by centrifugation on Triosil/Ficoll. The HL-A antisera had been selected within the London Transplant Group for typing renal homograft donors and recipients. Eighteen well-defined antigens were studied: HL-A1, 2, 3, 9, 10, 11 and 28 at the first locus, and HL-A5, 7, 8, 12, 13, 14, 17, 27, W10, W15, and W22 at the second locus. All patients and normal subjects were also tested with antisera detecting the W5 and/or W18 antigens. In addition, 42 of the patients and 75 of the controls were tested for the W29 and Da25 components of the W19 antigenic group. The 4c antigen group was taken as that detectable by antisera reacting with both HL-A5 and W5/W18 antigens. Only the HL-A5 antigen showed a significant difference in frequency between the two groups; it showed a frequency of 15.9% among patients, and 3.6% among controls. Group 4c antigens were also more common among patients (31.7%) than controls (15.6%). Whether this difference can be accounted for by the presence of non-HL-A specificities is uncertain.

2118 ANOTHER OBSERVATION OF INCREASED FREQUENCY OF HL-A 5 AND HLA-A 8 IN HODGKIN'S DISEASE. (Eng.) Perlin, E. (Natl. Naval Med. Cent., Bethesda, Md.): O'Donnell, M. *Am. J. Clin. Pathol.* 64(2):277-278; 1975.

A prospective comparison was made of the frequencies of histocompatibility (HL-A) antigens 5, 8, and 11 in 47 patients (47 Caucasian, one Negro) with Hodgkin's disease and in 155 normal controls (153 Caucasian, two Negroes). HL-5 and HL-A 8 occurred, respectively in 18.8% and 25.0% of the patients with Hodgkin's disease compared with 5.2% and 9.1% of the controls. These differences between the patient and control groups were statistically significant. HL-A 11 did not occur more frequently in Hodgkin's disease patients (2.1%) than in controls (9.7%). This study provides further evidence for the association of HL-A 5 and Hodgkin's disease, and supports the existence an association of HL-A 8.

2119 PROLONGED SURVIVAL IN BRONCHOGENIC CARCINOMA ASSOCIATED WITH HL-A ANTIGENS W-19 AND HL-A5: A PRELIMINARY REPORT. (Eng.) Dellon, A. L. (Natl. Cancer Inst., Bethesda, Md. 20014); Rogentine, G. N., Jr.; Chretien, P. B. *J. Natl. Cancer Inst.* 54(6):1283-1286; 1975.

The HL-A antigens were determined retrospectively in a group of 14 surgically cured bronchogenic carcinoma patients and prospectively in another group of 100 untreated patients. Of the cured group, nine had squamous carcinoma, and five had adenocarcinoma; of the untreated group, 38 had squamous carcinoma, 32 had adenocarcinoma, 20 had oat cell carcinoma,

and 10 had undifferentiated carcinoma. HL-A typing was done with antisera defining 21 specificities. In the retrospective group, the frequencies of antigens W-19 and HL-A5 were significantly increased when compared with the noncancer control and the prospective lung cancer populations. In the latter group, 60% of the patients with W-19 and 58% with HL-A5 survived without evidence of tumor for at least one yr after treatment compared with 15% of patients with neither of these antigens. This preliminary study suggests that the presence of HL-A antigens W-19 and HL-A5 confers resistance to dissemination of bronchogenic carcinoma.

2120 THE EFFECT OF SOLUBLE ANTIGENS FROM POLYOMA TUMOR AND LIVER CELLS ON STIMULATION OF LYMPH NODES MEASURED BY INCORPORATION OF ^{14}C-LEUCINE. (Eng.) Porwit-Bóbr, Z. (ul. Grodzka 52, 31-044 Krakow, Poland); Radziejowska, A.; Slowik, M.; Stanisz, A. *Arch. Immunol. Ther. Exp. (Warsz.)* 23(2):257-261; 1975.

Immunological responses of cultured lymph node cells from CBA mice to polyoma tumor cell antigens, liver antigens, and phytohemagglutinin (PHA) were studied. The different tissue antigens were prepared by extraction with 3 M KCl, followed by dialysis of the extract against 0.15 M NaCl, centrifugation of the dialyzed preparation, and 10-fold concentration of the supernatant. The different lymph node cell cultures were prepared with cells separated from the nodes by teasing with forceps, followed by filtration. The stimulation index was determined on the basis of incorporation of ^{14}C-leucine in short-term cultures of the cells. The results obtained with normal lymph node cells were compared with those obtained with lymph node cells from tumor bearers. The stimulation index was lower in lymphocytes derived from tumor bearers than in those derived from normal donors; this suggests inhibition of protein synthesis in the former case, because incorporation of leucine represents protein synthesis. The blastoid responses to polyoma-associated antigens and PHA were similar, in contrast to the high response to liver-associated antigens. Since liver-associated antigens contain more sugar components that have affinity for the corresponding binding sites on the lymphocyte membrane, it is concluded that the high stimulation by liver antigens may be connected with their different structure (mainly of carbohydrate components).

2121 ANTIGENICITY OF CARCINOGEN AND VIRAL INDUCED SARCOMAS IN INBRED AND RANDOM BRED GUINEA PIGS. (Eng.) Forni, G. (Microbiological Associates, Bethesda, Md.); Rhim, J. S.; Pickeral, S.; Shevach, E. M.; Green, I. *J. Immunol.* 115(1):204-210; 1975.

Tests were made for tumor specific transplantation antigen (TSTA) in methylcholanthrene (MCA)-induced and Kirsten strain mouse sarcoma virus (Ki-MSV)-induced guinea pig tumors. An MCA-induced sarcoma, tumor 574, originated in a strain 13 guinea pig. Ki-MSV-induced sarcomas, tumors 543 and 880, originated in a strain 13 and a strain 2 guinea pig, respectively. The MSV-induced tumors were nonproductive for virus. Syngeneic guinea pigs were injected sc with irradiated cells in complete Freund's adjuvant. Immunized and control animals were challenged 20 days after immunization with viable tumor cells injected sc. Tests for serum antibody were carried out by cytotoxicity and immunofluorescence procedures using cultured cells as targets. Thirty days after immunization with the MCA-induced tumor, only 10% of the test animals still bore a progressively growing tumor, compared with 100% of control unimmunized animals. In contrast, neither strain 2 nor strain B MSV tumor-immunized animals showed any protection or increased resistance to tumor cell growth. No tumor specific antibodies were detectable in syngeneic guinea pigs immunized with any of the tumors. Transplantation of MCA- and MSV-induced tumors in unimmunized allogeneic animals showed additional differences between the tumors, with regard to the presence or absence of guinea pig alloantigens B, C, D, and E in the test animals. MSV-induced tumor 880 grew equally well in the syngeneic inbred strain 2, in allogeneic inbred strain 13, and in random-bred 13$^+$, B$^+$ animals. However, in 13$^+$, D$^+$ random-bred animals, the tumors were rejected. Thus, the ability to transplant this tumor is correlated with the presence of the B antigen in the recipient animal. This and additional findings indicated that the B alloantigen plays a central role in acting as a guinea pig transplantation antigen. In all cases, the absence of B antigens in the recipient led to tumor rejection and anti-B antibody protection.

2122 DIFFERENCES BETWEEN SOLUBLE COMPLEMENT-FIXING ANTIGENS DERIVED FROM P3HR-1, RAJI, AND Hk-Ly-28 CELL LINES. (Eng.) Weliky, N. (Syst. Group T.R.W., Redondo Beach, Calif.); Leaman, D. H., Jr.; Kallman, B. J. *J. Natl. Cancer Inst.* 54(3):593-596; 1975.

Soluble complementing-fixing antigens of the Epstein-Barr virus-producing P3HR-1 Burkitt lymphoma cell line, the virus non-producing RAJ1 Burkitt lymphoma cell line, and the Hk-Ly-28 nasopharyngeal cancer cell line were isolated, identified by use of viral capsid antigen-positive human sera, and characterized according to differences in their stability to chemical and physical conditions. The soluble antigen was prepared by freezing and thawing the cells three times and sonicating at 18,000 cycles/sec for 3-30-sec intervals. The supernatant taken after centrifugation at 2,000 x *g* was frozen and recentrifuged; the supernatant was frozen. The complement-fixing activity did not change in the soluble fraction upon further centrifugation. Microcomplement-fixation tests were performed using the soluble antigen diluted by a factor of two with 0.05 M Veronal in saline or Oxoid BR-16 tablets dissolved in 0.1% gelatin. Human serum was diluted from 1:4 to 1:128 and 1.7 U of guinea pig complement was added. The viral capsid antigen/early antigen titers of the two fluorescent-labeled antibody-positive, complement-fixing-positive human sera used in these tests was 160/10 and 320-640/<10, resp. The viral capsid antigen/early antigen titer of the fluoroscene-labeled antibody-negative, comple-

ment-fixing-negative serum was <2/<10. P3HR-1, RAJ1, and Hk-Ly-28 soluble antigen fractions, diluted or undiluted, were unaffected by dialysis against 0.05 M Veronal at 4-6 C for at least three days, and by dialysis against unbuffered saline at pH 5.8-6.8 for at least 18 hr. The P3HR-1 soluble complement-fixing antigen lost titer when heated or exposed to acid perchlorate under conditions in which RAJ1 and Hk-Ly-28 soluble antigens were stable. The losses of titer of the P3HR-1 soluble complement-fixing antigen suggest the following: either the major antigenic determinants of the RAJ1 soluble complement-fixing antigen, stable under the same conditions, are not the major soluble form of complement-fixing antigen in the P3HR-1 cell; or, if they are present, the structure of the molecular species of which they are a part has been so altered that the antigen has become susceptible to heat and acid perchlorate. These results also have a bearing on the selection of conditions for the development of identification tests and purification procedures for complement-fixing antigens of Burkitt's lymphoma, nasopharyngeal cancer and other Epstein-Barr virus-associated tumors which could lose activity if subjected to purification or fractionation steps, including dialysis or dilution.

2123 SOMATIC HYBRID OF THYMUS LEUKEMIA (+) AND (-) CELLS FORMS THYMUS LEUKEMIA ANTIGENS BUT FAILS TO UNDERGO MODULATION. (Eng.) Liang, W. (La Rabida-Univ. Chicago Inst., Ill.); Cohen, E. P. *Proc. Natl. Acad. Sci. USA* 72(5):1873-1877; 1975.

A somatic hybrid of ASL-1 leukemia cells [II-2^a, thymus leukemia (TL)1,2,3, Thy-1b] and LM(TK)$^-$ cells [II-2^k, TL(-), Thy-1(-), thymidine kinase deficient] was formed with the aid of inactivated Sendai virus. The II-2 antigens of both parental cells were formed in approximately equivalent amounts by the hybrid cells, and they possessed a hybrid karyotype. As determined by five independent experimental procedures (antibody and complement-mediated cytotoxicity tests, the reduction of specific antibody activity of antiserum of known titer, immunofluorescent tests, mixed hemagglutination tests, and their direct isolation), TL antigens but not Thy-1 antigens were formed by the hybrid cells. TL antigens of the hybrids failed to undergo modulation under conditions leading to the modulation of TL antigens of parental ASL-1 cells. Modulation was attempted with TL 1,3, TL 2, or TL 1,2,3 antisera of high titer. Hybrid cells were incubated for up to 30 hr in medium with TL antisera. Both direct and indirect immune methods were attempted. These results indicate that genetic mechanisms controlling the expression of TL antigens may be distinguished from the capacity of the cells to undergo modulation.

2124 IMMUNOGENETICS OF A THYMUS ANTIGEN IN LYMPHOMA-PRONE AND LYMPHOMA-RESISTANT COLONIES OF WILD MICE. *(Eng.)* Blankenhorn, E. P. (Div. Biol., California Inst. Technol., Pasadena); Gardner, M. B.; Estes, J. D. *J. Natl. Cancer Inst.* 54(3):665-672, 1975.

Two populations of wild mice were examined for the presence of *Thy-1* antigen, the frequency of its alleles, and the development of spontaneous lymphomas in order to determine if the spontaneous lymphomas observed were related to the *Thy-1*^{a} and *Thy-1*^{b} alleles and were of T-cell origin. Direct cytotoxicity towards lymphoid cells and indirect absorption of antiserum were measured using a trypan blue dye exclusion technique. Viable spleen cells from healthy young (< 18 months) or old (> 18 months) wild mice were *Thy-1* positive (15-55% destroyed in the direct cytotoxicity assay). Wild mice from the population resistant to lymphoma development were homozygous for the *Thy-1*^{b} allele, whereas mice from the lymphoma prone population were polymorphic for *Thy-1*^{a} and *Thy-1*^{b} alleles but had a higher frequency of *Thy-1*^{b} allele. Alleles at other loci on chromosome 9 (*Mod-1*^{a} and *Trf*^{b}) were studied and found to be fixed in both wild mouse populations. *Thy-1* antigen could not be detected on spleen cells from 80% of the lymphomatous mice, although *Thy-1* antigen was found in brain tissue from these mice. The thymus of mice with lymphoma did not appear to be involved in the lymphomatous proliferation. The results indicate that *Thy-1* antigens are present in the two wild mouse strains studied. The authors suggest that the spontaneous lymphoma seen in one of the populations is derived from cells of nonthymus origin.

2125 SERUM ALPHA-FETO PROTEIN (α-FP) AND HEPATOMA IN RHODESIAN AFRICANS. (Eng.) Seggie, J. (Dept. Medicine, Univ. Rhodesia, Salisbury, Rhodesia); Gelfand, M. *Trans. R. Soc. Trop. Med. Hyg.* 69(2):209-211; 1975.

Sera of 30 African patients with histologically proven hepatocarcinoma were tested by countercurrent electrophoresis for the presence of α-fetoprotein. α-Fetoprotein was detected in the serum of only 14 (46.7%) of the 30 patients. There was no sex difference, seven males and seven females being positive for α-fetoprotein. There was no relationship to age, the patients' ages ranging from 26-70 yr. Although a positive test for serum α-fetoprotein is strong evidence in favor of a diagnosis of hepatocarcinoma, these results indicate that a negative test is of little value. Thus, all patients with suspected hepatocarcinoma should be subjected to liver biopsy, with the aid of a liver scan where the liver is diffusely enlarged.

2126 *IN VITRO* IMMUNOLOGICAL STUDIES ON EAST AFRICAN CANCER PATIENTS. II. INCREASED SENSITIVITY OF BLOOD LYMPHOCYTES FROM UNTREATED BURKITT LYMPHOMA PATIENTS TO INHIBITION OF SPONTANEOUS ROSETTE FORMATION. (Eng.) Gross, R. L. (Western General Hosp., Crewe Road, Edinburgh, EH4 2XU, Scotland); Levin, A. G.; Steel, C. M.; Singh, S.; Brubaker, G.; Peers, F. G. *Int. J. Cancer* 15(1):132-138; 1975.

The nonimmune rosette-forming capacity of peripheral blood lymphocytes from patients with Burkitt's lymphoma (BL) was studied. The nonimmune SRBC rosette formation is a marker for thymus-dependent lymphocytes (T cells). Using cells recovered from lympho-

cyte banks, collected at various stages of BL, this technique was applied to material from 14 children with the disease and from 12 control children of comparable age, either healthy or suffering from a nonmalignant disorder. The lymphocytes were originally separated from defibrinated venous blood by centrifugation over a Ficoll/triosil layer. In addition to the SRBC rosette test, rosette-inhibition tests were carried out in which the lymphocytes were preincubated with anti-lymphocyte globulin. Using the SRBC rosette test, a significant reduction in the proportion of rosette-forming lymphocytes was found in samples from untreated BL patients. After remission was induced, the difference between BL patients and controls was abolished. Rosette formation was more readily inhibited by anti-lymphocyte globulin in samples from untreated BL patients than in controls. Upon the induction of remission, rosette inhibition shifted toward the control values. Since lymphoid cells from BL biopsies often have an antibody coating, and since tumor cells are of lymphoid origin, it appears reasonable that anti-tumor antibody may cross-react with normal circulating lymphocytes. Such a situation would explain the present findings. Thus, the findings were compatible with the presence of a circulating factor, which impairs the capacity of T-cells to form rosettes with SRBC, in untreated BL patients.

2127 LOCALIZATION OF AGGREGATED CELL SURFACE ANTIGENS OF TARGET CELLS BOUND TO CYTOTOXIC T LYMPHOCYTES. (Eng.) Berke, G. (Weizmann Inst. Science, Rehovot, Israel); Fishelson, Z. *J. Exp. Med.* 142(4):1011-1016; 1975.

The redistribution of aggregated cell surface antigens of target cells bound to cytotoxic T lymphocytes was investigated. It was found that cap formation induced by antibody always occurred toward the site of binding. In some cases, the fluorescent cap concentrated exclusively between the cells and in others it was in close proximity to the conjugation area. The localization of the cap at the binding site of CTL and target cells may indicate that binding occurs through a unique area of the target cell where the cap tends to localize. Alternatively, the localization of the cap may be a consequence of CTL-target cell interaction, which attracts a high local concentration of target cell surface determinants to the site of the binding. It is suggested that the polar localization of capped target cell surface determinants is a consequence of cytotoxic T-lymphocyte (TL) target cell interaction.

2128 T LYMPHOCYTE DIFFERENTIATION *IN VITRO* IN ATAXIA TELANGIECTASIA ASSOCIATED WITH LYMPHOSARCOMA. (Eng.) Boumsell, L. (Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021); Incefy, G. S.; Bernard, A.; Schwartz, S.; Smithwick, E.; Good, R. A. *J. Pediatr.* 87(3):435-438; 1975.

Markers for T lymphocytes and *in vitro* differentiation of peripheral blood lymphocytes (PBL) and bone marrow cells under the influence of human thymic extract were studied in a 9-yr-old girl with ataxia telangiectasia. The patient had a lymphosarcoma. Serum IgM levels were markedly elevated but IgG, IgA, and IgE levels were normal. Among the PBL, 45% of the cells were positive for human T-lymphocyte antigen (HTLA), and 35% formed spontaneous large rosettes with SRBC. Both values fell within the lower values for normal sera studied by the same procedure. For PBL separated on bovine serum albumin (BSA) gradient, cells converted from HTLA- to HTLA+ phenotype were located in layer III, whereas an increased number of E rosettes appeared in layer IV. For the normal control, an increased number of both markers was observed in layers I and IV. For bone marrow cells separated on BSA gradient, cells converted to HTLA+ phenotype were situated in layer I and II, whereas an increased number of E rosettes was observed only in layer I for the patient. For the normal control, an increased number of both markers occurred in layer I. The abnormality of development of T and B cells present in ataxia telangiectasia was not revealed by this investigation. Future studies of the disease should focus on patients with extreme deficits of either T-cell markers or T-cell functions and/or serum IgA and IgE levels.

2129 COMPARATIVE STUDIES ON THE REACTIVITY OF T-CELLS AND NON-T-CELLS IN LYMPHOCYTE TRANSFORMATION EXPERIMENTS AND CYTOTOXICITY TESTS. (Eng.) Warnatz, J. (Univ. Hosp. Erlangen-Nürnberg, West Germany); Scheiffarth, F.; Zenker, W. *Cell Immunol.* 17(2):517-524; 1975.

Defined populations of lymphocytes from immunized and nonimmunized mice were tested for antigen recognition and for lymphocytotoxicity. Simian virus 40 (SV40)-transformed fibroblasts of Balb/c mice (SV40TF) were used for immunization of syngeneic mice and for target cells in the cytotoxicity tests. To prepare immune cells, 10^7 SV40TF were injected first into footpads of Balb/c and nu/nu Balb/c mice, then five times i.p. at three weekly intervals. For preparation of antiserum, 10^6 SV40TF were injected into Balb/c mice at eight weekly intervals. Unfractionated cells were obtained by removal of RBC on a Ficoll-isopaque gradient. A preparation composed mainly of T-cells was obtained by passing unfractionated T-cells through a nylon wool column. A mainly non-T-cell preparation was recovered from cells retained on the nylon wool column, the cells being subsequently treated with anti-θ-serum and complement. Purified T-cells were obtained by passing the RBC-free T-cell preparation through an Ig-anti-Ig column. A subpopulation of non-T-cells was obtained by passing the non-T-cell preparation through an Ig-anti-Ig coated column. Antigen recognition was based on lymphocyte transformation as determined by DNA synthesis (tritiated thymidine uptake) of lymphocytes in the presence of SV40TF. Lymphocytotoxicity was carried out by a microcytotoxicity test using lymphocyte fractions and SV40TF target cells. The immune lymphocytes were significantly stimulated by SV40TF and were highly cytotoxic to SV40TF. Experiments with isolated fractions of lymphocytes showed that these reactions were attributed mainly to T-cells. Non-T-cell preparations, including the subpopulation of Ig-receptor-lacking non-T-cells, were, however, also activated by SV40TF to moderately increased DNA synthesis and were cyto-

toxic to SV40TF. Non-T-cells from normal Balb/c mice were not stimulated by SV40TF as effectively as were lymph node and spleen cells of *in vivo* primed nu/nu mice, indicating that in spite of the T-cell defect, nude mice show antigenic recognition of SV40TF. Although lymph node and spleen cells from immunized nude Balb/c mice were stimulated to DNA synthesis by SV40TF, they were barely cytotoxic to the target cells. The cytotoxicity of non-T-cells, but not of T-cells, from immunized or nonimmunized mice was increased in the presence of target cell specific antibody; this is interpreted as a coating of target cells by antibody, which allowed contact of killer B-cells and target cells.

2130 THYMUS-DERIVED LYMPHOCYTES IN PATIENTS WITH SOLID MALIGNANCIES. (Eng.) Potvin, C. (Natl. Cancer Inst., Bethesda, Md.); Tarpley, J. L.; Chretien, B. *Clin. Immunol. Immunopathol.* 3(4): 476-481; 1975.

Peripheral blood levels of T cells were measured in 94 patients with operable tumors (32 adenocarcinomas, 16 melanomas, 12 sarcomas, and 22 head and neck and 12 pelvic squamous carcinomas), in 25 patients with advanced or recurrent tumors, in 125 patients treated surgically five or more years earlier (21 adenocarcinomas, 15 melanomas, ten sarcomas and 48 head and neck and 31 pelvic squamous carcinomas) and in 240 healthy volunteers, using a spontaneous lymphocyte rosette assay. Patients with clinically operable melanoma, sarcoma or squamous carcinoma and patients with advanced malignancies had significantly lower T cell levels than normal and adenocarcinoma patients. Normal T cell levels were seen in patients considered cured of adenocarcinoma, melanoma or squamous carcinoma. Significantly lower T cell levels were seen in irradiated head and neck squamous carcinoma patients than in nonirradiated patients. No such difference was seen in patients cured of pelvic squamous carcinoma. The authors suggest that the spontaneous lymphocyte rosette assay may be clinically useful as a correlate of cell-mediated immunity.

2131 STIMULATION OF MIXED LYMPHOCYTE CULTURES AND CYTOTOXIC RESPONSES: EVIDENCE THAT T CELLS EXPRESS SD BUT NOT LD ANTIGENS, WHEREAS B CELLS EXPRESS BOTH. (Eng.) Simpson, E. (Clin. Res. Cent., Harrow, England). *Eur. J. Immunol.* 5(7): 456-461; 1975.

A method of separating mixed lymphocyte response (MLR) antigens from H-2K and H-2D antigens by fractionation of spleen cells is reported. A variety of strains of inbred and hybrid mice were used in the study. Spleen cell suspensions from which erythrocytes were removed by hemolysis were used as responder or as stimulator "unseparated spleen" cells. T cell enrichment was accomplished by incubation of unseparated spleen cells on nylon wood colums, followed by slow elution; B cell enrichment, by treatment of unseparated spleen cells with anti-Thy 1.2 antiserum and guinea pig complement. Before cultures were set up, the stimulator cells were irradiated from a ^{60}Co source. MLR and cell-mediated lympholysis (CML) cultures were set up in parallel, using 0.5×10^6 responder cells and the same number of irradiated stimulator cells in 0.2 ml volumes of complete RPMI 1640 medium. On day 3 or 4 of culture, the MLR cultures were pulsed with ^{3}H thymidine, harvested 4-6 hr later, and measured for radioactivity. On day 5, the CML cells (attacker cells) were harvested and used in a cytotoxicity assay involving ^{51}Cr-labeled EL-4 (C57BL/10 ascites tumor), P-815 (DBA/2 ascites tumor), or YAC (A strain ascites lymphoma) cells as target cells. For the cytotoxicity test, 1×10^5 target cells were mixed with 4×10^5, 2×10^5, or 1×10^5 attacker cells in MEM/10% fetal calf serum, the mixtures were incubated, and the supernatants were used for gamma counting. A typical experiment used the BALB/c anti-(C57BL/10 x BALB/c)F_1 combination in which the responder cells were nylon column-enriched splenic T cells. It was found that MLR and CML cells are generated in responder T cell populations by B but not by T stimulator cells in a wide variety of strain combinations. It is suggested that MLR-stimulating (LD) antigens are expressed on B but not T cells, and that their presence is necessary in order to generate cytotoxic responses to H-2K and/or H-2D (SD) antigens which are present on both T and B cells. The finding that B cells and macrophages stimulate MLR is consistent with the hypothesis that Ia antigens, expressed on both of these cell types but not on nylon column-purified T cells, are the LD antigens. The need for two signals, one provided by the LD antigens, the other by the SD antigens, to generate cytotoxic cells was shown by the finding that, although splenic T cells possess the SD antigens, they fail to stimulate cytotoxic responses.

2132 DISPARITY OF MIXED LYMPHOCYTE REACTIVITY TO CULTURED CELLS OF HUMAN T AND B LYMPHOID LINES. *(Eng.)* Pauly, J. L. (Dep. Med. B, Roswell Park Mem. Inst., Buffalo, N.Y.); Minowada, J.; Han, T.; Moore, G. E. *J. Natl. Cancer Inst.* 54(3):557-562; 1975.

One-way mixed lymphocyte reactivity of peripheral blood lymphocytes (PBL) from healthy donors was measured towards cultured lymphoid cell (CLC) lines of T cell origin (RPMI 8402 and MOLT-4) and B cell origin (RPMI 8382, 8392, 8412, 8422, and 8442) in order to compare the reactivity of PBL to cells of different origin. Lymphocyte reactivity was measured using ^{3}H-thymidine incorporation into PBL. Using a ratio of PBL-to-CLC of 1:1, all of the CLC lines of B cell origin induced high levels of reactivity, whereas neither of the two CLC of T cell origin did. Changing the PBL-to-CLC ratio to 1:20 had no effect on reactivity towards the T lymphoid cells. The difference in reactivity was also seen when the T and B CLC lines were irradiated or arrested with mitomycin C. Treatment of the PBL with *Vibrio cholerae* neuraminidase (VCN) enhanced their reactivity towards the B-CLC but not towards the T-CLC. Addition of irradiated RPMI 8402 cells did not affect the reactivity of PBL to mitogens or B lymphoid CLC. The results indicate that the different reactivity of PBL to CLC of B and T cell origin is not due to different kinetics of interaction, and is not humorally mediated. It is sug-

gested that this property is a characteristic intrinsic to the T cell lines studied.

2133 "B" CELL ORIGIN OF MALIGNANT CELLS IN A CASE OF AMERICAN BURKITT'S LYMPHOMA. CHARACTERIZATION OF CELLS FROM A PLEURAL EFFUSION. (Eng.) Binder, R. A. (Georgetown Univ. Hosp., 3800 Reservoir Road NW, Washington, D. C. 20007); Jencks, J. A.; Chun, B.; Rath, C. E. *Cancer* 36(1):161-168; 1975.

Whether tumor cells from a pleural effusion of a 49-year-old white male American patient with Burkitt's lymphoma were derived from T or B lymphocytes was determined. The lymphoma cells were separated from the effusion by centrifugation on a Ficoll-Hypaque gradient and suspended in complete RPMI 1640 culture medium. Tests for T cells were based on rosette formation with sheep SRBC; tests for B cells, on immunofluorescence staining with fluorescein-labeled antisera monospecific for human IgG, IgA, and IgM. Ninety-eight percent of the cells showed membrane fluorescence to antihuman IgM, while only 2% showed formation of rosettes with sheep RBC. Further, there were no cells showing membrane fluorescence to anti-IgG or anti-IgA. It was concluded, therefore, that the Burkitt cells in the malignant effusion were derived from B cells. The finding agrees with that previously reported for an African patient. Epstein-Barr virus antibody levels were absent in the American patient, but this was not unique and could not be used to exclude the diagnosis of Burkitt's lymphoma. It is suggested that the morphological identity of tumor cells from African and American varieties of the disease may represent similar responses to different oncogenic stimuli.

2134 DELAYED CUTANEOUS HYPERSENSITIVITY AND PERIPHERAL LYMPHOCYTE COUNTS IN PATIENTS WITH ADVANCED CANCER. (Eng.) Lee, Y.-T. N. (Univ. South. California Sch. Med., Los Angeles); Sparks, F. C.; Eilber, F. R.; Morton, D. L. *Cancer* 35(3): 748-755; 1975.

The prognostic value of delayed cutaneous hypersensitivity (DCH) responses to common skin antigens and to 2,4-dinitrochlorobenzene (DNCB) in 183 patients with advanced and nonlymphomatous solid cancers was investigated. Absolute peripheral lymphocyte counts were studied and an inter-relation with reactivity to both recall and to new antigens was noted. Patients were followed for at least six months or until death. Histologic tumor types studied were: melanoma (65), sarcoma (28), squamous cell carcinoma (23), and adenocarcinoma (67). Patients were challenged with 100 μg of DNCB on the day of sensitization and with doses of 100, 50, and 25 μg 14 days after sensitization. Skin tests were examined after 48 hr. Along with DNCB challenge, each patient was tested for DCH to four skin antigens: *Monilla* (2 U), mumps (2 U), tuberculin PPD (5 U intermediate strength), and streptokinase-streptodornase (Varidose, Lederle, 10 U). The disease progression rate within six months of testing was lower in patients who responded positively to a challenge dose of 50 μg DNCB. Reactivity to recall antigens had prognostic value only in patients with adenocarcinomas. Those patients with adenocarcinomas who reacted strongly to DNCB and one or more skin test antigen had the best prognosis, while those who were nonreactive to all had the worst prognosis (cancer progression rate, 18% *versus* 78%). Peripheral lymphocyte counts were related to DCH and DNCB skin test results; the presence or absence of lymphocytopenia (count < $1000/mm^3$) had prognostic value in patients with positive skin tests. In such patients, the disease progression rate was much higher in those anergic to DNCB who were lymphocytopenic (90% *versus* 40%). This suggests that DCH to DNCB, recall antigens, and peripheral lymphocyte counts are useful immunologic measurements in patients with advanced cancer. The prognostic value of these tests is increased when multiple tests are employed.

2135 CELLULAR RECOGNITION AND ACTIVATION WITHIN THE LYMPHOID SYSTEM. *(Eng.)* Kersey, J. H. (Dep. Lab. Med. Pathol., Univ. Minnesota, Minneapolis); Booth, G. J. *Am. J. Clin. Pathol.* 63(5):629-635; 1975.

Cell pairings of human phagocytes and lymphocytes were studied to determine intracellular interactions with similar and dissimilar cells. WBC rich plasma was incubated with a carbamyl-iron-latex suspension to label phagocytes, prior to incubation for one hr in fetal calf serum media. Fifty pairs of cells in contact were examined in each experiment. Lymphocyte subpopulations were characterized in purified lymphocyte preparations using the sheep RBC (SRBC) rosette assay. Only 2-5% of the total cells used formed pairs or larger aggregates. The results showed that phagocytes combine preferentially with phagocytes, and that lymphocytes combine with lymphocytes in greater proportions than predicted by random association. Likewise, B lymphocytes tend to pair more often with B lymphocytes, and T lymphocytes tend to pair preferentially with T lymphocytes. The author assigns a possible role of the lymphoid system in the recognition and destruction of cancerous cells.

2136 EFFECTS OF HUMAN LYMPHOCYTES ON CULTURED NORMAL AND MALIGNANT CELLS. (Eng.) Pierce, G. E. (Univ. of Kansas Medical Center, Kansas City, Kans. 66103); DeVald, B. L. *Cancer Res.* 35(7):1830-1839; 1975.

A modified mictocytotoxicity assay was used to test the effects of lymphocytes from 76 normal subjects and from 144 cancer patients against normal and neoplastic target cells *in vitro*. The study was divided into three sequential time phases: lymphocytes were separated by a Ludox-polyvinylpyrrolidone technique in the first phase, by nylon wool filtration in the second phase, and by a Ficoll-Hypaque technique in the third phase. In the first two phases, normal lymphocytes were just as frequently toxic against tumor target cells as were lymphocytes of cancer patients, and both normal and patient lymphocytes

were frequently toxic to fibroblasts. In the third phase, toxicity was observed in 45% (91/200) of patient lymphocyte-tumor target combinations compared with 16% (30/191) normal lymphocyte-tumor target combinations. However, an approximately equal frequency of reactivity was noted against normal target cells; i.e., toxic reactions were observed in 25% (24/96) of patient lymphocyte-fibroblast combinations and in 20% (19/94) normal lymphocyte-fibroblast combinations. The different results obtained in this phase cannot be attributed solely to the different method of lymphocyte separation; other factors, such as better growth status of the target cells and greater facility in performing the assays, might also be responsible. Results of the last phase raise the possibility that both specific and nonspecific reactions may contribute to the toxicity observed with the lymphocytes of cancer patients against tumor cells Before microcytotoxicity can be widely used to monitor specifically the tumor-immune response of patients, factors responsible for nonspecific reactions must be clearly defined and eliminated.

2137 AGGLUTINATION OF LEUKEMIC AND 2,4-DINITROPHENYL-TAGGED NORMAL HUMAN LYMPHOCYTES BY WHEAT GERM AGGLUTININ. *(Eng.)* Madyastha, P. R. (Univ. Kansas Med. Cent., Kansas City); Barth, R. F.; Madyastha, K. R. *J. Natl. Cancer Inst.* 54(3):597-600; 1975.

Normal human lymphocytes, 2,4-dinitrofluorobenzene (DNFB)-tagged normal lymphocytes, and human leukemic lymphocytes obtained from four patients with acute lymphocytic leukemia (ALL), from three patients with chronic lymphocytic leukemia (CLL) and from cultured ALL cells were incubated with wheat germ agglutinin (WGA). Agglutination was maximal when a ratio of 10^{11} molecules DNFB/cell was used for tagging. There was a marked difference in agglutination between normal lymphocytes and leukemic or DNFB-tagged lymphocytes, the former group being more resistant to agglutination at WGA concentrations between 50-800 μg/ml. Agglutination of normal lymphocytes by 200 μg/ml WGA was completely inhibited by 0.001 M *N*-acetylglucosamine (GlcNAc) while agglutination of DNFB-tagged normal lymphocytes and leukemic lymphocytes was completely blocked by 0.1 M GlcNAc. Leukemic cells tagged with DNFB did not demonstrate increased agglutinability compared to untagged leukemic cells. The results suggest that the similarity in agglutination properties of DNFB-tagged normal lymphocytes and leukemic lymphocytes is due to the binding of the WGA lectin to specific receptors rather than to DNFB residues.

2138 INTRINSIC LYMPHOCYTE DEFECT IN HODGKIN'S DISEASE: ANALYSIS OF THE PHYTOHEMAGGLUTININ DOSE-RESPONSE. (Eng.) Ziegler, J. B. (Sch. Med., Univ. New South Wales, Australia); Hansen, P.; Penny, R. *Clin. Immunol. Immunopathol.* 3(4):451-460; 1975.

Delayed hypersensitivity to candida, mumps, streptokinase/streptodornase and PPD antigens was assessed in 27 newly diagnosed, untreated Hodgkin's disease patients, and was found to be of no use as an indicator of staging. Four out of 14 patients in stages 1 to 3A and eight out of 13 patients in stage 3B or 4 were anergic. Peripheral blood lymphocytes from the same patients were cultured with phytohemagglutinin (PHA) concentrations of 0-800 μg/ml and stimulation to blast formation was measured by ^{3}H-thymidine uptake. Initial peripheral lymphocyte counts were reduced in ten out of 13 stage 3B or 4 patients and were normal in all stage 1 to 3A patients. PHA responses were reduced in 12 out of 13 stage 3B or 4 patients and in four out of 14 stage 1 to 3A patients. All of the lymphopenic stage 3B or 4 patients showed reduced PHA response; in seven of them, it was seen at all PHA concentrations used. Four of the seven patients had normal lymphocyte counts. The authors suggest that the lymphocyte dose-response to PHA is a sensitive index of the defect in the lymphocytes of Hodgkin's disease patients and that it may be useful in clinical staging. The results indicate that, prior to the appearance of lymphopenia, a functional defect in PHA response is frequently found.

2139 LYMPHOCYTE RESPONSE TO PHYTOHEMAGGLUTININ (PHA) IN *IN VITRO* CULTURE AND QUANTITATIVE AND QUALITATIVE CHANGES IN SERUM IMMUNOGLOBULINS IN PATIENTS WITH PLASMA-CELL MYELOMA. (Eng.) Traczyk, Z. (Inst. Hematol., Warsaw, Poland); Cieśluk, S.; Śnigurowicz, J. *Arch. Immunol. Ther. Exp. (Warsz.)* 23(1):105-112; 1975.

To determine if the capability of lymphocytes to undergo blastic transformation (BTL) in phytohemagglutinin (PHA)-stimulated cultures is related to changes in immunoglobulin (Ig) synthesis, 27 patients with plasma-cell myeloma and one patient with Waldenstrom's macroglobulinemia were studied. Blood samples from 40 healthy donors served as controls. The homogeneous protein fraction of serum Ig was analyzed by paper and immunoelectrophoresis, dilution immunodiffusion, and immunochromatography and was assayed quantitatively by radial immunodiffusion. Fifty percent of the patients with plasma-cell myeloma demonstrated impaired BTL capability of lymphocytes. The degree of BTL depression was not correlated with the patient's clinical status or therapy. In addition, it was not related to the class of monoclonal protein, its serum concentration, or to lower serum levels of other Ig. In patients with type χ monoclonal protein, BTL was impaired in two of three cases. In patients with monoclonal protein type λ, only one of eight cases showed BTL impairment. The results indicate that the defect in the response of lymphocytes to PHA *in vitro* in these patients lies within the cells, and not in extracellular factors.

2140 DEPRESSION IN LYMPHOCYTE RESPONSE TO GENERAL MITOGENS BY OWL MONKEYS INFECTED WITH *HERPESVIRUS SAIMIRI*. *(Eng.)* Wallen, W. C. (Litton Bionetics, Inc., Kensington, Md.); Rabin, H.; Neubauer, R. H.; Cicmanec, J. L. *J. Natl. Cancer Inst.* 54(3):679-685; 1975.

Twelve adult owl monkeys were injected with *Herpes-*

virus saimiri (HVS) to determine the effects of HVS infection on lymphocyte response to concanavalin A (Con A), phytohemagglutinin (PHA) and pokeweed mitogen (PWM). The animals were inoculated i.m. and s.c. with 1.0 ml HVS. Samples of peripheral blood lymphocytes (PBL) were studied immunologically, virologically and hematologically. Lymphocyte cultures (1 x 10^6 cells/ml) were incubated with 2.0 and 20 μg PHA, 1.0 and 10 μg Con A, or 4 and 40 μl PWM. PBL response to mitogens and DNA synthesis were measured by ^{3}H-thymidine incorporation. Six monkeys developed lymphoma and lymphocytic leukemia, and two developed lymphoma only. PBL of these eight animals showed the presence of HVS as determined by an infective center assay using VERO cell cultures, as early as four weeks following inoculation. Reduced response to PHA, Con A and PWM was seen in all monkeys that developed leukemia. Increasing PHA concentrations (500, 100, 50, 10, 5, 1 and 0.1 μg/culture) did not increase incorporation of label, indicating that the loss of reactivity was not due to a shift in dose-responsivity. Five of the six monkeys showed greatly increased DNA synthesis which corresponded to HVS recovery from PBL. The two animals with lymphoma only, showed altered mitogen response at 12 and 18 weeks after inoculation, but not before then. Two HVS-infected monkeys developed chronic infections but showed no signs of neoplasia. Altered mitogen reactivity was observed in one of these animals. The last two monkeys died of unrelated causes, and showed no signs of altered mitogen reactivity, or evidence of HVS in their PBL. The reactivity of these animals suggests that the loss of PBL response to mitogens may be related to the development of neoplastic disease, and not only due to HVS infection.

2141 TUMOR DEVELOPMENT AFTER POLYOMA INFECTION IN ATHYMIC NUDE MICE. (Eng.) Stutman, O. (Mem. Sloan Kettering Cancer Cent., New York, N.Y.). *J. Immunol.* 114(4):1213-1217; 1975.

Tumor development induced by polyoma virus infection (strain LID-1) in athymic nude (nu/nu) mice and normal control nu/+ or CBA/H mice was studied to determine the role of cells of thymic origin (T-cells) in resistance to tumor development. Tumor incidence was 83 to 90% in nu/nu mice and 1 to 10% in controls when polyoma virus was injected at 15 and 30 days of age. Tumor incidence in nu/nu mice decreased to 50% and 25% in 60 and 120 day old mice, resp. I.p. grafting with normal thymuses from CBA/HT6T6 newborn mice provided partial resistance, but not if the grafts were enclosed in diffusion chambers which prevented cell release. Treatment of the normal spleen cells *in vitro* with anti Thy.1.2 antiserum and rabbit C had no effect on resistance transfer; anti-mouse Ig and C treatment eliminated the transfer. Pretreatment of the donor mice with 25 mg/kg of cyclophosphamide had no effect on the transfer, but 100 mg/kg abolished it. Spleen cells from 15-day-old nu/+ mice also provided partial resistance to tumor development which was abolished by *in vitro* treatment with anti-Ig or anti-Thy.1.2. Virus concentrations were assayed in nu/nu and nu/+ mice five and 15 days after infection and were found to be increased in nu/nu mice at five days, but equal at 15 days. Hemagglutination-inhibition antibody titers in the sera of nu/+ and nu/nu mice were the same 15 to 20 days after virus infection, but were higher in nu/+ mice 30 to 35 days after infection. The results indicate that resistance transfer imparted by normal nu/+ mouse thymuses may be mediated by T cells, but that B cells also play a role. Hemagglutination-inhibition serum antibody titers and viral replication do not explain the differences seen in polyoma oncogenesis.

2142 TUMORIGENICITY OF VIRUS-TRANSFORMED CELLS IN *NUDE* MICE IS CORRELATED SPECIFICALLY WITH ANCHORAGE INDEPENDENT GROWTH *IN VITRO*. (Eng.) Shin, S.-I. (Albert Einstein Coll. Medicine, Bronx, N.Y. 10461); Freedman, V. H.; Risser, R.; Pollack, R. *Proc. Natl. Acad. Sci. USA* 72(11):4435-4439; 1975.

Clonal isolates of mouse 3T3 cells and primary rat embryo cells, harvested nonselectively after infection by simian virus 40 (SV40), were tested for tumorigenicity in the immune-deficient *nude* mice (a+/nu stock on a BALB/c background). The cellular growth properties *in vitro* associated with neoplastic growth *in vivo* were observed. In addition, mouse 3T3 cells transformed by murine sarcoma virus (MuSV, Kirsten strain), and revertants isolated from cells fully transformed by either SV40 or MuSV were also studied. The single cellular property consistently associated with tumorigenicity in *nude* mice is the acquisition by virus-transformed cells of the ability to proliferate *in vitro* in the absence of anchorage. Anchorage-independent growth was measured by colony-farming ability in methyl cellulose. Other cellular parameters of virus-induced transformation, such as lack of sensitivity to high cell density and the capacity to grow in low serum concentration, were dissociable from cellular tumorigenicity. Essentially identical results resulted from analogous experiments with four rat-embryo-cell clones. Specific selection *in vivo* for tumorigenic cells from anchorage-dependent mouse cells resulted in the isolation of anchorage-independent cells. Conversely, a single-step selection *in vitro* for anchorage-independent cells from non-tumorigenic cells resulted in a simultaneous selection of highly tumorigenic subclones.

2143 MACROPHAGE-MEDIATED DESTRUCTION OF HUMAN MALIGNANT CELLS *IN VIVO*. *(Eng.)* Mansell, P. W. A. (Tulane Univ. Sch. Med., New Orleans, La.); Ichinose, H.; Reed, R. J.; Krementz, E. T.; McNamee, R.; Di Luzio, N. R. *J. Natl. Cancer Inst.* 54(3):571-580; 1975.

Seven patients with malignant melanoma, one patient with adenocarcinoma of the breast and one patient with adenosquamous carcinoma were studied in order to evaluate the effects of intralesion injections of glucan and plasma macrophage "recognition factor" (RF) on tumor growth *in vivo*. The heparin-precipitated human RF fraction (20 mg/ml saline) and glucan (100 mg/g lipid emulsion base) were prepared; s.c. metastases of all patients were then

injected with glucan, glucan and RF, RF alone, saline, or lipid emulsion. Plasma RF activity was measured before, during and after injections using the uptake of ^{131}I-triolein-labeled lipid emulsion by rat liver slices. Glucan, either alone or in combination with RF, caused resolution of the tumors in as short as five days. Resolution appeared to be complete in small tumors but was limited in the larger ones. Treatment with RF alone resulted in necrosis and regression in size in seven of ten tumors. Histologic studies showed that glucan induced local tumor necrosis resulting in sterile abscess formation. The abscess exudate was composed primarily of macrophages, degenerating neutrophils and tumor cell fragments. The macrophages were confined to the walls of the abscesses and to the exudate. After all types of treatment, the plasma RF levels rose in all the patients. Cessation of treatment of patients with advanced disease was accompanied by a fall in plasma RF. The results indicate that glucan and RF intralesional injections result in macrophage-mediated cytotoxicity directed against the tumor cells.

2144 THE IMMUNOLOGY OF MALIGNANT MELANOMA. (Ger.) Schieferstein, G. (Univ.-Hautklinik, D-7400 Tubingen, West Germany). *Klin. Wochenschr.* 53(6):241-255; 1975.

2145 AUTOIMMUNE HEMOLYTIC ANEMIA. (Eng.) Dacie, J. V. (Royal Postgraduate Medical Sch., London, England). *Arch. Intern. Med.* 135(10):1293-1300; 1975.

2146 VIRAL EXPRESSION, ONCOGENICITY, AND ANTIGENICITY OF A MOUSE SALIVARY GLAND TUMOR AND TWO CELL LINES DERIVED FROM IT. (Eng.) Castelli, L. (Regina Inst. Cancer Res., Viale Regina Elena n. 291, Rome, Italy); Neri, G.; Frigola, A.; Di Fava, F. M.; Giuliano, M. C. *Cancer Res.* 35(9);2394-2402; 1975.

2147 POTENTIATION OF HAMSTER TUMORS BY NORMAL CELLS OR CHARCOAL. (Eng.) El Mishad, A. M. (Baylor Coll. Medicine, Houston, Tex. 77025); McCormick, K. J.; McCormick, N. K.; Trentin*, J. J. *Cancer Res.* 35(8):2098-2103; 1975.

2148 COMPARATIVE GROWTH OF BOVINE LYMPHOSARCOMA CELLS AND LYMPHOID CELLS INFECTED WITH *THEILERIA PARVA* IN ATHYMIC (NUDE) MICE. (Eng.) Irvin, A. D. (East African Veterinary Res. Organization, Muguga, PO Box 32, Kikuyu, Kenya); Brown, C. G. D.; Kanhai, G. K.; Stagg, D. A. *Nature* 255(5511):713-714; 1975.

2149 THE ROLE OF ALLOANTIBODIES ON *IN VIVO* GROWTH OF ALLOGRAFTED TUMOR. (Eng.) Yutoku, M. (Immunology Res. Dept., Roswell Park Memorial Inst., Buffalo, N.Y. 14203); Kitagawa, M. *Transplant. Proc.* 7(2):243-246; 1975.

2150 TRANSPLANTATION BEHAVIOR OF A.TH AND A.TL T-CELL LYMPHOMAS IN CONGENIC RESISTANT AND HYBRID STRAINS. (Eng.) Goding, J. W. (Royal Melbourne Hosp., Victoria 3050, Australia); Warner, N. L. *J. Exp. Med.* 142(2): 536-541; 1975.

2151 RENAL TRANSPLANTATION AND CANCER. THE SCANDIA TRANSPLANT MATERIAL. (Eng.) Birkeland, S. A. (Odense Univ. Hosp., Odense, Denmark); Kemp, E.; Hauge, M. *Tissue Antigens* 6(1):28-36; 1975.

2152 IMMUNE RESPONSE AGAINST APPARENTLY HOST-COMPATIBLE TRANSPLANTABLE TUMORS. (Eng.) Manson, L. A. (Wistar Inst., Philadelphia, Pa.); Goldstein, L.; Thorn, R.; Palmer, J. *Transplant. Proc.* 7(2):161-164; 1975.

2153 PHYTOHEMAGGLUTININ-STIMULATED IMMUNE RESPONSE: ASSAY IN COLORECTAL CARCINOMA PATIENTS. (Eng.) Kaplan, M. S. (Dept. of Surgery, Univ. of California, 5901 E. Seventh St., Long Beach, Calif.); Mino, F. O.; Kummerfeld, K. B.; Lundak, R. L. *Arch. Surg.* 110(10):1217-1220; 1975.

2154 LOCAL ADOPTIVE TRANSFER OF THE ANTITUMOR CELLULAR IMMUNE RESPONSE IN SYNGENEIC AND ALLOGENEIC MICE STUDIED WITH A RAPID RADIOISOTOPIC FOOTPAD ASSAY. (Eng.) Takeichi, N. (Natl. Cancer Inst., Bethesda, Md.); Boone, C. W. *J. Natl. Cancer Inst.* 55(1):183-187; 1975.

2155 CARRIER SPECIFICITY *VS* HAPTEN SPECIFICITY IN CLASSICAL CELL-MEDIATED CONTACT SENSITIVITY TO DINITROCHLOROBENZENE. (Eng.) Levis, W. R. (Dermatology Branch, Natl. Cancer Inst., Bethesda, Md. 20014); Powell, J. A.; Whalen, J. J. *J. Immunol.* 115(4):1170-1172; 1975.

2156 CELL-MEDIATED IMMUNITY AGAINST ALLOGENEIC TUMOR AFTER *IN VITRO* DEPLETION OF HISTOCOMPATIBILITY REACTIVE CELLS. (Eng.) Lukic, M. L. (Tufts Univ. Sch. Medicine, 136 Harrison Ave., Boston, Mass. 02111); Leskowitz*, S. *Proc. Soc. Exp. Biol. Med.* 148(2):420-423; 1975.

2157 CELL-MEDIATED ANTITUMOR IMMUNITY IN BREAST CANCER PATIENTS EVALUATED BY ANTIGEN-INDUCED LEUKOCYTE ADHERENCE INHIBITION IN TEST TUBES. (Eng.) Grosser, N. (Dept. Medicine, McGill Univ., Montreal, Quebec, Canada); Thomson*, D. M. P. *Cancer Res.* 35(9):2571-2579; 1975.

2158 RESPONSE TO PRIMARY INFECTION WITH *HERPESVIRUS SAIMIRI* IN IMMUNOSUPPRESSED JUVENILE AND NEWBORN SQUIRREL MONKEYS. (Eng.) Martin, L. N. (Tulane Univ., Delta Regional Primate Res. Center, Covington, La. 70433); Allen, W. P. *Infect. Immun.* 12(3):528-535; 1975.

2159 IMMUNOSUPPRESSIVE EFFECT OF PREGNANCY-ASSOCIATED $ALPHA_2$-GLYCOPROTEIN. (Eng.) Than, G. N. (Univ. Medical Sch., 7624 Pecs, Edesanyak utja 17, Hungary); Csaba, I. F.; Karg, N. J.; Szabo, D. G.; Ambrus, M.; Bajtai, G. *Lancet* 2(7933):515; 1975.

2160 IMMUNOSUPPRESSION BY SPLEEN CELLS FROM MOLONEY LEUKEMIA. COMPARISON OF THE SUPPRESSIVE EFFECT ON ANTIBODY RESPONSE AND ON MITOGEN-INDUCED RESPONSE. (Eng.) Cerny, J. (Harvard Sch. Public Health, 665 Huntington Ave., Boston, Mass. 02115); Stiller, R. A. *J. Immunol.* 115(4):943-949; 1975.

2161 IMMUNOSUPPRESSION EFFECTED BY MACROPHAGE SURFACES. (Eng.) Ptak, W. (Dept. Clinical and Experimental Immunology, Sch. Medicine, Cracow, Poland); Gershon, R. K. *J. Immunol.* 115(5):1346-1350; 1975.

2162 SERUM POTENTIATION OF GRANULOCYTE AND MACROPHAGE COLONY FORMATION *IN VITRO*. (Eng.) Metcalf, D. (Royal Melbourne Hosp. P.O. 3050 Victoria, Australia); MacDonald, H. R.; Chester, H. M. *Exp. Hematol.* 3(4):261-273; 1975.

2163 IMMUNOFLUORESCENT STAINING OF HUMAN LYMPHOCYTES FOR THE DETECTION OF SURFACE IMMUNOGLOBULINS. (Eng.) Preud'homme, J. L. (Hosp. St-Louis, 75475 Paris Cedex 10, France); Labaume, S. *Ann. N.Y. Acad. Sci.* 254:254-261; 1975.

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2165 A HUMAN LYMPHOID CELL LINE WITH AN IgG-LIKE MEMBRANE COMPONENT. (Eng.) Nilsson, K. (Wallenberg Lab., Univ. of Uppsala, Box 562, S-751 22 Uppsala, Sweden); Ghetie, V.; Sjoquist, J. *Eur. J. Immunol.* 5(8):518-526; 1975.

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2175 MIGRATION OF PERIPHERAL LEUKOCYTES IN THE PRESENCE OF CARCINOEMBRYONIC ANTIGEN. STUDIES IN PATIENTS WITH CHRONIC INFLAMMATORY DISEASES OF THE INTESTINE AND CARCINOMA OF THE COLON AND PANCREAS. (Eng.) Straus, E. (Mount Sinai Sch. Medicine of City Univ. of New York, N.Y. 10029); Vernace, S.; Janowitz, H.; Paronetto, F. *Proc. Soc. Exp. Biol. Med.* 148(2):494-497; 1975.

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2177 SEPARATION OF A TUMOUR SPECIFIC TRANSPLANTATION-TYPE ANTIGEN FROM THE ASCITIC FLUID OF MICE BEARING A SYNGENEIC LYMPHOMA. (Eng.) Wolf, A. (Chester Beatty Res. Inst., Clifton Ave., Belmont, Sutton, Surrey, England); Steele, K. A. *Br. J. Cancer* 31(6):684-688; 1975.

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2193 *IN VITRO* STUDIES ON HUMAN B AND T CELL PURIFIED POPULATIONS: STIMULATION BY MITOGENS AND ALLOGENEIC CELLS, AND QUANTITATIVE BINDING OF PHYTOMITOGENS. (Eng.) Clot, J. (Laboratoire de Bacteriologie, Virologie et Immunologie, Hopital Saint-Eloi, Montpellier, France); Massip, H.; Mathieu, O. *Immunology* 29(3):445-453; 1975.

2194 MORPHOLOGY, SURFACE MARKERS, AND *IN VITRO* RESPONSES OF A HUMAN LEUKEMIC T CELL. (Eng.) Insel, R. A. (Center for Disease Control, Public Health Service, Atlanta, Ga. 30333); Melewicz, F. M.; La Via, M. F.; Balch, C. M. *Clin. Immunol. Immunopathol.* 4(3):382-391; 1975.

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See also:

* (Rev): 1808, 1809, 1835, 1836, 1837, 1838, 1847
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2198 CANCER OF THE BILE DUCTS ASSOCIATED WITH ULCERATIVE COLITIS. (Eng.) Akwari, O. E. (Mayo Clin. Found., Rochester, Minn.); Van Heerdan, J. A.; Foulk, W. T.; Baggenstoss, A. H. *Ann. Surg.* 181(3):303-309; 1975.

Thirteen patients with bile duct cancer (excluding the gallbladder) and associated ulcerative colitis were studied. Of 32,300 autopsies between 1935 and 1973, 0.37% revealed bile duct cancer. Of this percentage, 1.6% were associated with ulcerative colitis. The mean age of the patients at onset of ulcerative colitis was 19 yr, with an average of 27.8 months duration of symptoms to the diagnosis of chronic ulcerative colitis. The onset in all patients was marked by severe diarrhea. In seven patients the diarrhea was frequently associated with severe hemorrhaging. Skin lesions occurred in five patients, including three with pyoderma gangrenosum. Most patients developed a pattern of mild-to-moderate disease with exacerbations and remissions. Various regimens aimed at symptomatic relief and support included proctocolectomy and total colectomy. The mean age of onset of bile duct cancer was 38 yr, representing an average of 19 yr from onset of ulcerative colitis symptoms to onset of symptoms referable to bile duct cancer. The previous mode of management of colitis had no specific effect on the pattern of disease leading to bile duct cancer. The onset was insidious in all cases, characterized by fatigue, weight loss, and anorexia, followed by development of acholic stools, dark urine, pruritus, and progressive jaundice; the most common initial physical findings were icterus and hepatomegaly. Surgical procedures included exploration, palliative tumor resection, duct-enteric anastomosis bypass, common duct exploration and T-tube decompression, and curative resection. Adjuvant chemotherapy and/or high dose radiation therapy was administered to several patients. The mean length of survival was 18.8 months from the onset of symptoms; this commonly followed a pattern of hepatic failure, sepsis, shock, renal shutdown, and death.

2199 PRIMARY INTESTINAL LYMPHOMA WITH PARAPROTEINEMIA. (Eng.) Shahid, M. J. (Am. Univ. Hosp., Beirut, Lebanon); Alami, S. Y.; Nassar, V. H.; Balikian, J. B.; Salem, A. A. *Cancer* 35(3):848-858; 1975.

The immunoglobulin patterns in five cases of primary intestinal lymphoma are described using electrophoresis with the LKB system. The main clinical features include abdominal pain, diarrhea and weight loss. Laparotomy reveals mesenteric node enlargement with malignant lymphoma and dilatation of small bowel. Malabsorption with low levels of serum protein, albumin, cholesterol and calcium are characteristic. There is dense infiltration of the lamina propria layer with plasma cells, lymphocytes and reticular cells. This polymorphic picture becomes more atypical in the deeper mucosa showing histocytes and giant cells. The plasma cell infiltration is postulated to be responsible for the malabsorption. Immunoelectrophoresis showed an abnormal elongated arc of precipitation extending throughout the alpha-2 and beta globulin region. The abnormal globulin is devoid of light chains. It is suggested that this IgA heavy chain disease is a variant of primary intestinal lymphoma, and not a separate entity.

2200 SYMPTOMATIC LIPOMAS OF THE GASTROINTESTINAL TRACT. (Eng.) Ackerman, N. B. (Dept. Surgery, Univ. Missouri-Kansas City, Kansas City, Mo.); Chughtai, S. Q. *Surg. Gynecol. Obstet.* 141(4):565-568; 1975.

Eleven patients (eight women, three men) with symptomatic lipomas of the gastrointestinal tract were observed between 1966 and 1972. The case reports of three patients are presented. The lipomas were generally relatively large, and the signs and symptoms consisted mainly of abdominal pain and chronic blood loss. These lesions most commonly were seen in the colon and in the region of the ileocecal valve, and were less commonly seen in the small intestine, stomach and esophagus. Distinguishing these tumors from carcinomas or sarcomas can be difficult, and these patients were generally in the same age range as those with cancer. Roentgenologic contrast studies are helpful in localizing the tumors, but accurate tissue diagnosis usually is not made until the lesions are excised. Operative management by either local excision or segmental resection is required, and the prognosis is excellent.

2201 PATHOLOGY OF ANGIOSARCOMA OF THE LIVER AMONG VINYL CHLORIDE-POLYVINYL CHLORIDE WORKERS. (Eng.) Thomas, L. B. (Natl. Cancer Inst., Bethesda, Md. 20014); Popper, H. *Ann. N.Y. Acad. Sci.* 246:268-277; 1975.

Gross and microscopic features of the hepatic angiosarcoma found in 13 vinyl chloride (VC) and/or polyvinyl chloride (PVC) workers are reported. Liver sections were obtained at autopsy from 9 of 10 patients who had died, and other samples were provided by needle or surgical biopsy. The average weight of the livers of seven of the nine patients who had died of angiosarcoma was 4,236 g, with a range of 1,560-7,300 g; all were massively involved with cystic, blood-filled tumors which replaced most of the liver. The principal microscopic features of the lesions included areas of hemorrhage, necrosis, and marked fibrosis, plus irregular infiltration of angiosarcoma into adjacent hepatic tissue. The angiosarcomas showed several structural patterns. The sinusoidal pattern was observed in 11 of 13 specimens. Sinusoidal, papillary and cavernous growth patterns apparently represented progressive changes due to continued proliferation and growth of the sarcomatous sinusoidal lining cells. Another histologic feature was an enlargement of the space of Disse. A few of the angiosarcomas had nodular areas of solid tumor composed of more anaplastic sarcoma cells. Deposits of angiosarcoma in the lungs, myocardium, epicardium, kidney, peritoneum, muscularis of the

small intestine, mesentery, mesenteric lymph nodes, and retroperitoneum were noted in one patient who also had massive liver involvement. The series of changes in the liver appears multicentric, with only some lesions progressing to fully developed angiosarcomas. The authors conclude that chronic exposure to VC, or perhaps some metabolic product of VC, caused stimulation and proliferation of both the parenchymal and stromal cells of the liver.

2202 ALPHA-ANTITRYPSIN DEFICIENCY AND HEPATIC CARCINOMA. (Eng.) Schleissner, L. A. (Harbor General Hosp., 1000 West Carson St., Torrance, Calif. 90509); Cohen, A. H. *Am. Rev. Respir. Dis.* 111(6):863-868; 1975.

The case report of a 49-yr-old man with homozygous $ZZ\alpha_1$-antitrypsin (AAT) deficiency and severe pulmonary emphysema with mixed hepatocellular and cholangiolar carcinoma in a noncirrhotic liver is presented. Pathologic findings at death, limited to the lungs and liver, included severe panlobular and bullous emphysema, moderate passive congestion of the liver, and a mass (6 cm diameter) deep within the central portion of the right lobe. The mass was surrounded by several smaller satellite lesions. PAS positive globules (an outstanding feature of the liver in AAT deficiency) were present in normal and malignant hepatocytes. The tumor pattern was biphasic. In the hepatocellular phase, the tumor was continuous with the hepatocyte cords and was composed of trabeculae and nests of polygonal cells. In the cholangiolar phase, the cells were arranged in acini and resembled proliferating bile ducts. Because of the increasing frequency of hepatomas in patients with AAT deficiency, it is suggested that a causal relation may exist.

2203 PERSISTENCE OF VINYL CHLORIDE-INDUCED LIVER INJURY AFTER CESSATION OF EXPOSURE. (Eng.) Berk, P. (Natl. Inst. Arthritis, Metabolism, Digestive Diseases, Natl. Inst. Health, Bethesda, Md. 20014); Martin, J. F.; Waggoner, J. G. *Ann. N.Y. Acad. Sci.* 246:70-77; 1975.

The case report of a 30-yr-old male Caucasian, and a follow-up of the apparent vinyl chloride-induced liver injury 2.5 yr after removal from exposure, is presented. The man was employed as a vinyl chloride (VC) polymerization tank cleaner for 5.5 yr. He presented initially with an undefined illness characterized by fever, weight loss, hepatosplenomegaly and mild elevations of serum bilirubin, SGOT and BSP retention. Diagnostic laparotomy revealed multiple areas of fine nodularity and fibrosis of the liver and an enlarged spleen. Liver biopsy revealed portal fibrosis and histologic sections of the spleen showed lymphoid and reticuloendothelial proliferation. At the follow-up examination, complete blood count, chest x-ray, liver scan, and all routine tests were normal. However, pulmonary function studies showed evidence of restrictive pulmonary disease, and subsequent to peritoneoscopy, extensive portal fibrosis was again found. Plasma disappearance studies of unconjugated radiolabeled bilirubin, indocyanine green, and cholyl glycine were undertaken and the latter was suggested to be the most sensitive indicator of hepatic dysfunction. Hepatic histology initially included normal lobular architecture, expansion of fibrosis of the portal areas, and bile duct proliferation; the follow-up peritoneoscopy revealed marked expansion and fibrosis of the portal tract, an increase in both mononuclear inflammatory cells and bile ducts, but the activated hepatocytes and sinusoidal lining previously noted was lacking. The normal values observed for many tests of liver function accompanied by a significant histologic lesion indicate the need for adequate screening of VC workers. This also reflects the fact that the principal anatomic lesion in VC-associated liver disease is fibrosis, with relative sparing of the hepatocytes. Parameters reflecting various aspects of collagen metabolism may provide useful screening tests.

2204 MALIGNANT MESENCHYMOMA OF THE HEART. (Eng.) Nagamine, Y. (Yamaguchi Univ. Sch. Med., Ube, Japan); Sasai, K.; Sasaki, K. *Acta Pathol. Jpn.* 25(2):241-249; 1975.

An autopsy case is presented of a 34-yr-old woman with malignant cardiac mesenchymoma. The patient had presented with cough, dyspnea, palpitation and orthopnea; the abdomen was slightly distended with ascites, and the lower extremities were edematous. Chest X-ray had disclosed enlargement of the heart, but no area of radio-opacity was found within the cardiac shadow. Death ensued four months later, and the autopsy revealed a tumor arising in the atrial septum, bulging into the left atrium. The tumor had metastasized to the left lung. Histologically, the tumor in the left lung resembled that of the more differentiated part of the cardiac tumor. This tumor had fibrosarcomatous, chondrosarcomatous, rhabdomyosarcomatous, and osseous elements. A theory of metaplastic origin of the mesenchymoma is possible, although this cannot be supported by these results.

2205 MALIGNANT MELANOMA OF THE CHOROID AS RELATED TO COEXISTENT BENIGN NEVUS. (Eng.) Arnesen, K. (Ulleval Sykehus, Norway); Nornes, M. *Acta Ophthalmol. (KBH.)* 53(2):139-152; 1975.

Malignant choroidal melanoma was studied using 95 human eyes, enucleated between 1960 and 1974. Emphasis was placed on coexistence of benign nevus, histological myelomal type, and prognosis. Benign nevus was found to be associated with 78% of all melanomas. Fifty-six percent of all melanomas were of the spindle cell type (according to Callender's classification). Benign nevus was associated with 83% of the spindle-type melanoma while only 71% of other types showed benign nevus. The prognosis tended to be better when elements of benign nevus were found at the base of the tumor than when they were not present. The results support the hypothesis that most cases of malignant choroidal melanoma

riginate from preexisting benign nevus, and that he tumors undergo a gradual change from a differ-ntiated to a less differentiated type.

206 TRANSITIONAL CELL CARCINOMA OF PROSTATE. (Eng.) Tannenbaum, M. (Columbia-Presbyt. ed. Cent., New York, N.Y.) *Urology* 5(5):674-678; 975.

eriurethral or transitional cell carcinoma of the rostate is a distinct histologic type occurring in rom 2-4% of prostate carcinomas. A review of the iterature dealing with this type of lesion is pre-ented and its morphological and clinical implica-ions are discussed. Histological patterns of inter-st to the clinician and pathologist include transi-ional cell hyperplasia; columnar surface cells sur-ounding a lumen; mitoses in invasive, undifferen-iated forms; *in situ* carcinomas, which can be non-nvasive of the fibromuscular stroma; a comedo pat-ern; and basal or reserve cells surrounding transi-ional cells. The author concludes that the impor-ance of this distinction lies in the assignment of herapy, and notes that transitional cell carcinoma f the prostate appears to be hormonally unresponsive

207 THYROID CANCER: TWENTY YEARS' EXPERIENCE IN A GENERAL HOSPITAL. (Eng.) Campbell, . J. (Selly Oak Hosp., Birmingham, England); Sage, . H. *Br. J. Surg.* 62(3):207-214; 1975.

ifty-four primary thyroid malignancies treated be-ween 1953 and 1973 are discussed. During this eriod, 1,058 thyroidectomies were performed, with 5 being confirmed as primary carcinomas. In 21 atients, a correct diagnosis of rapidly progressive naplastic tumors was possible on clinical grounds lone. Thirty-three carcinomas were viewed with arying degrees of clinical suspicion, and it is mportant to note the percentage of each group that s likely to be malignant: solitary nodule, 2.7%; ontoxic multinodular goiter, 12.8%; nontoxic diffuse oiter, 1.3%; all nontoxic goiters, 8.7%; and toxic oiter, 0.04%. The results of treatment of female atients were considerably better than those of the ale patients, with the age of presentation exerting marked influence on survival upon both sexes. athological and epidemiological studies confirmed e biological individuality of the various histo-gical types of the carcinoma that were presented many forms with great concern over those who pre-nted with a form often indistinguishable from that benign goiter. Unilateral thyroid cancers have monstrable intraglandular dissemination, which may plain the transformation of malignant solitary nod-e into a multinodular form; the latter presented average 12 yr earlier. The prognosis of any can-r is determined primarily by its histological type d clinical stage. This study emphasizes the im-rtance of clinical staging of the primary tumor. inically obvious carcinomas carried uniformly bad ognoses, being invariably anaplastic, because dif-rentiated carcinomas reached this stage far less equently. The slower growth of differentiated ncer resulted in presentation at an earlier stage, which suggests that they pass through solitary nodule and multinodular forms before presenting the picture of a frankly malignant locally invasive goiter. These results emphasize the value of clinical staging when applied to thyroid cancer and clearly show that increasing 'T number' is accompanied by worsening of prognosis.

2208 MYOEPITHELIOMA OF MINOR SALIVARY GLAND ORIGIN: LIGHT AND ELECTRON MICROSCOPICAL STUDY. (Eng.) Stromeyer, F. W. (Tripler Army Med. Cent., Honolulu, Hawaii); Haggitt, R. C.; Nelson, J. F.; Hardman, J. M. *Arch. Pathol.* 99(5): 242-245; 1975.

A gingival tumor involving the anterior maxilla was removed from a 14-yr-old boy and studied by light and electron microscopy. Tissues from the second of two previous incisional biopsies were examined by light microscopy only. Therapeutic external irradiation (Cobalt 60) had been given prior to excision. The histological appearance of the excised tumor was unchanged from that of the earlier biopsy specimen, and confirmed the analysis of a poorly differentiated epithelial neoplasm of undetermined origin. Electron microscopy confirmed the general size and shape of the nuclei and cytoplasm. Uniformly dispersed microfilaments, 50-100 Å in diameter, were seen arranged in parallel streams or in a herringbone pattern. By light microscopy, many of the cells had eccentric nuclei and abundant cytoplasm; they resembled the myoepithelial cells of mixed tumors of the salivary glands and of a previously reported myoepithelioma. A myoepithelial cell origin, suggested by the tumor's proximity to mucous salivary glands, was confirmed by electron microscopy. Examination of the second biopsy showed all of the features of myoepithelioma, except focal condensations of filaments. Several features of this case, including cellular pleomorphism, mitotic figures, and invasion of bone, indicate a low grade malignant potential. The authors suggest that myoepitheliomas may be related to mixed tumors of salivary glands, although this case did not exhibit the epithelial or mesenchymal elements essential to mixed tumors. This may be due to the immature nature of the cells, or to a reduced capacity for metaplasis on the part of minor salivary glands.

2209 AN EXPERIMENTAL MOUSE TESTICULAR TERATOMA AS A MODEL FOR NEUROEPITHELIAL NEOPLASIA AND DIFFERENTIATION: II. ELECTRON MICROSCOPY. (Eng.) Herman, M. M. (Stanford Univ. Sch. Medicine, Stanford, Calif. 94305); Sipe, J. C.; Rubinstein, L. J.; VandenBerg, S. R.; Spence, A. M.; Vraa-Jensen, J. *Am. J. Pathol.* 81(2):421-444; 1975.

The electron microscopic features of the stages of divergent neuroepithelial differentiation in the solid implants of a transplantable mouse testicular teratoma (OTT-6050) are presented and compared to the sequential stages of cytogenesis that have been described in the developing avian and mammalian CNS. Primitive neuroepithelial tumor cells showed the

features of undifferentiated multipotential matrix (or ventricular) cells of the neural tube. They formed primitive medullary rosettes, from which various transitions were traced to more differentiated, cilia-containing ependymoblastomatous rosettes; the transitional features included increased granular endoplasmic reticulum and microvilli formation. Glial differentiation was characterized by the presence of mature ependymal rosettes and of astrocytes containing glial filaments. Neuronal differentiation included the development of synapses and the presence of dense-core vesicles in nerve cell processes. No intermediate cell forms were found that suggested multiple lines of differentiation occurring within a single cell. The demonstration of neuronal, astrocytic, and ependymal differentiation in areas contiguous to undifferentiated neuroepithelial cells confirms that neoplastic neurocytogenesis can be studied in this experimental tumor system.

2210 INVESTIGATIONS ON THE CARCINOGENIC BURDEN BY AIR POLLUTION IN MAN. XII. EARLY PATHOLOGICAL ALTERATIONS OF THE BRONCHIAL EPITHELIUM IN SYRIAN GOLDEN HAMSTERS AFTER INTRATRACHEAL INSTILLATION OF BENZO(a)PYRENE. 2. FURTHER ULTRASTRUCTURAL STUDIES. (Eng.) Reznik-Schuller, H. (Abteilung fur Experimentelle Pathologie, Medizinische Hochschule Hannover, W. Germany); Mohr, U. *Zentralbl. Bakteriol.* [*Orig. B.*] 160(2):108-129; 1975.

The ultrastructural alterations occurring in the bronchial epithelium of 32 female Syrian hamsters treated weekly with 0.63 mg benzo(a)pyrene administered intratracheally were studied. A control group of 18 animals was given only the dispensing solution, saline, dodecylsulfate, Tris-HCl and EDTA. Beginning in the fifth week, two experimental animals and one from the control group were sacrificed weekly. In the control group the bronchial epithelium retained its normal structure throughout the observation period of 20 wk. In the fifth week focal areas of cell proliferations with swollen mitochondria were observed. By the tenth week epithelial invaginations had become frequent. Increased numbers of and enlarged lysomes were observed in the weeks 8-10. Marked hyperplasia developed up to the 11th wk. By the 14th week epithelial cells had penetrated the basement membrane, and formations of epithelial cells were seen in the peribronchial lung tissue in close association with the alveolar epithelial cells. Between the 14th and 20th weeks these lesions gave rise to small bronchogenic adenomata, which consisted of ciliated and nonciliated cells and rare basal cells. In the marginal regions of the adenomata, ciliated cells frequently occurred possessing long cilia oriented toward adjacent lying aveolar epithelial cells, which they seemed to displace. The epithelial cells of the bronchi did not exhibit a keratinizing potency during the observation period. Squamous metaplasia was found only in the tracheal epithelium, possibly due to the short observation time. These studies confirm and expand upon previously published reports of ultrastructural alterations in the bronchial epithelium.

2211 ULTRASTRUCTURAL STUDIES OF H-1 PARVOVIRUS REPLICATION. I. CYTOPATHOLOGY PRODUCED IN HUMAN NB EPITHELIAL CELLS AND HAMSTER EMBRYO FIBROBLASTS. (Eng.) Singer, I. I. (Putnam Mem. Hosp. Inst. Med. Res., Bennington, Vt.); Toolan, H. W. *Virology* 65(1):40-54; 1975.

The morphogenesis and cytopathological effects of the parvovirus H-1 were studied by electron microscopy in parasynchronous cultures of SV40-transformed human newborn kidney epithelial cells (NB) and hamster embryo fibroblasts (HEF). Morphometry revealed that NB cells had 20% more H-1 particles/μm^2 of nucleus than the HEF; there were, however, ten times more DNA-containing virions in the hamster fibroblasts than in the human cells. Both cell types produced virus at comparable overall rates. At 12 hr postinfection (p.i.), the outstanding abnormal changes in both cell types occurred in their nucleoli. Fragmentation and loss of light and dark nucleolar fibrous components resulted in a vacuolation of the fibrous centers; high concentrations of H-1 particles without DNA centers (incomplete) formed compact linear arrays on the chromatinlike fibers remaining in these rarefied areas. In conjunction with the nucleolar alterations, fewer polysomes were observed in the cytoplasmic matrix and on the surface of the rough endoplasmic reticulum. At 18-36 hr p.i., a number of nuclei exhibited hollow spherules (0.5-3.5 μm in diameter) composed of fibrous and/or granular nucleolar elements that contained *incomplete* H-1 particles in their lumena or adherent to component fibers. These spherules appeared to be artifacts of nucleolar breakdown having little to do with normal H-1 development. Their association with empty virions may indicate an affinity between the nucleolus and H-1 proteins. Most virus, including the bulk of *complete* virions, was situated on *extranucleolar* chromatin fibers, forming linear or trabecular aggregates. As massive cellular degeneration took place (36 hr p.i.), the chromatin fibers appeared to condense, releasing the previously attached virus. High concentrations of H-1 particles were observed along the edges of large newly formed condensed chromatin masses located on the nuclear periphery; no virus was found *within* these masses. Also, conglomerates of liberated virions formed extensive paracrystalline arrays on the extracellular surfaces of intact plasmalemmata of adjacent cells or on fragments of disrupted membranes. Usually, each H-1 paracrystal was composed almost exclusively of either complete particles (even in NB cells which synthesize over 90% "empty" virus), or incomplete virions, indicating that differences may exist in their surface properties.

2212 AN ELECTRON MICROSCOPE STUDY OF "PACINIAN NEUROFIBROMA." (Eng.) Weiser, G. (Dept. Pathol., Univ. Innsbruck, Austria); *Virchows Arch.* [*Pathol. Anat.*] 366(4):331-340; 1975.

A "Pacinian neurofibroma" from the right flank area of an 11-day-old male infant was studied by light and electron microscopy. Unlike the usual neurofibroma, this was characterized by a proliferation of so-called perineurial cells. Groups of surface vesicles,

the absence of mesoaxons and a fragmented basal lamina differentiated these perineurial cells from Schwann cells. The formation of the perineurial cells could be traced continuously from small and wide submicroscopic cell bands, and club-shaped thickenings to ribbon-like cell complexes, as well as to tactile-like structures. The most developed complexes and structures, visible by light microscopy, do not correspond with real tactile corpuscles, but can be considered to be neoplastic structures of the perineurium. The Pacinian neurofibroma should be considered to be a neurofibroma with a dominant proliferation of perineurial cells.

2213 PLASMA MEMBRANE STRUCTURES OF MEDULLOBLASTOMA AND CEREBELLAR SARCOMA. (Eng.) Tani, E. (Hyogo Coll. Medicine, Nishinomiya, Mukogawa-cho, Hyogo, Japan); Mormura, T.; Kaba, K.; Higashi, N. *Acta Neuropathol. (Berl.)* 32(3):257-267; 1975.

Three medulloblastomas and one cerebellar sarcoma were studied by electron microscopy. The medulloblastomas were obtained at operation from the vermis and from between the cerebellar tonsils in children under ten years old. The cerebellar sarcoma was obtained from a boy of eight years and was seen in the meninges over the vermis as well as in the vermis and in the medial region of the right cerebellar hemisphere. The average number of membrane particles per square micrometer of plasma membrane was 710 on face A and 70 on face B of medulloblastoma and 1,280 on face A and 160 on face B of cerebellar sarcoma. The membrane particles were often aggregated in medulloblastoma and diffusely scattered in cerebellar sarcoma. Small gap junctions were occasionally found in cerebellar sarcoma and not evident in medulloblastoma. Round membrane protrusions, about 0.5-0.6 μ in diameter and provided with several small depressions on their foot, were often observed in region of narrow perinuclear cytoplasm of cerebellar sarcoma and different in structure from cytoplasmic processes. Although the limited number involved here does not allow a definite conclusion, there is an indication that the plasma membrane structures are different in medulloblastoma and cerebellar sarcoma.

2214 NEUROFIBROSARCOMA OF SPERMATIC CORD. (Eng.) Johnson, D. E. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.); Kaesler, K. E.; Mackay, B. M.; Ayala, A. G. *Urology* 5(5):680-683; 1975.

A neurofibrosarcoma arising within the spermatic cord is reported. A left inguinal orchiectomy with excision of the spermatic cord at the internal inguinal ring was performed on a 65-yr-old man. The surgical specimen contained a firm, irregular, multinodular tumor measuring 5 cm in greatest diameter. There was no capsule, but the margins appeared well demarcated from the surrounding cord structures. The cut section demonstrated a white, fibrous-like surface with rare areas of necrosis. Histologically, tumor sections revealed proliferation of spindle cells in no particular growth pattern. Sections from peripheral portions of the tumor showed well-differentiated malignant cells without significant pleomorphism, while the center revealed closely apposed spindle cells and numerous mitotic figures. By electron microscopy, tumor cells in longitudinal orientation showed "remarkable" attenuated cytoplasm. The cytoplasmic processes formed parallel bundles, and the limiting cell membranes of adjacent processes were apposed to one another. Cell contact specializations uniting cells and processes were identified as desmosomes, with few or no associated tonofilament bundles. Cell nuclei were elongated. Scattered mitochondria and short, irregular profiles of rough-surfaced endoplasmic reticulum were present in most cell processes. Microtubules occurred rarely, but slender, longitudinally oriented filaments were present in many processes. The sarcoma was intimately related to a nerve bundle and consisted of tumor cells of a histologic appearance and arrangement strongly suggestive of neurofibrosarcoma. The authors believe that neurogenic sarcomas can be distinguished by electron microscopy by the presence of specific fine structural features as well as by the absence of ultrastructural characteristics diagnostic of other type-specific sarcomas.

2215 IMMUNOLOGIC COMPETENCE OF REGIONAL LYMPH NODES IN PATIENTS WITH BREAST CANCER. (Eng.) Ellis, R. J. (Cornell Univ. Med. Coll., New York, N.Y.); Wernick, G.; Zabriskie, J. B.; Goldman, L. I. *Cancer* 35(3):655-659; 1975.

The degree of sensitization to breast cancer was compared between lymphocytes from ipsilateral lymph nodes and those of peripheral blood (PB) lymphocytes of 24 patients undergoing radical mastectomy. Inguinal lymph nodes and peripheral blood from ten patients undergoing open heart surgery served as controls. Using the *in vitro* assay of migration inhibition (MI), a greater immunologic response was observed with lymphocytes from regional lymph nodes (RLN), as compared to circulating lymphocytes, when challenged with breast cancer antigen. In the control group, MI was -10% with the RLN and -2% with PB lymphocytes. In the breast cancer group, MI was 21% for RLN, and 7% in the PB lymphocytes. A significant delayed hypersensitivity response (MI>20%) was found in 70.8% of the RLN in the mastectomy group as compared to 25% in the PB samples in the same group. These results support the concept that RLN in breast cancer patients are immunologically competent and are more reactive than circulating lymphocytes.

2216 ULTRASTRUCTURE OF CYTOPLASMIC HEMOSIDERIN INCLUSION BODIES IN MALIGNANT PHAGOCYTIC LYMPHOCYTES. (Eng.) Presentey, B. (Health Insurance Institution, Kupat Holim, Rehovot 76-100, Israel); Hod, I; Rosin, A. J. *Experientia* 31(3): 365-367; 1975.

Cytoplasmic iron-containing inclusions were detected in about 20% of peripheral lymphoid cells from a patient with lymphoma-leukemia. The inclusions reacted positively with methylene blue, toluidine

blue, Nile blue sulfate, and Prussian blue. The neutrophile granulocytes showed a very marked positive alkaline phosphatase reaction. Ultrastructural studies revealed the presence of medium and large lymphoid cells with undulated cytoplasmic membrane and irregular nuclear surface. Phagocytic vacuoles containing electron-dense material were regularly found in the lymphoid cells. That the inclusions were composed of hemosiderin was supported by electron micrographs showing characteristic ferritin granules. This study demonstrates that malignant lymphoid cells, like nonmalignant lymphocytes, possess phagocytic activity.

2217 EXPERIMENTAL INVESTIGATIONS ON CELL RESORPTION FROM THE PERITONEAL CAVITY BY USE OF THE SCANNING ELECTRON MICROSCOPE. (Ger.) Remmele, W. (Pathologisches Inst. der Stadtischen Kliniken, D-6200 Wiesbaden 1, Schwalbacher Str. 62, West Germany); Richter, I.-E.; Wildenhof, H. *Klin. Wochenschr.* 53(10):913-922; 1975.

The resorption of homologous RBC and Ehrlich ascites tumor cells from the peritoneal cavity was studied after ip injection in rats and mice. The injection of RBC and tumor cells was followed by the appearance of stomata in the peritoneal surface of the diaphragm, but not in the lateral abdominal wall, and absorption of cells through these stomata into the large lymphatic vessels was demonstrated. The findings indicate the probability of the intraperitoneal lymphohematogenous spread of tumor cells at least in the early stage of tumor infiltration of the peritoneum. This stage is followed by implantation of tumor cells on the peritoneum.

2218 NEEDLE-LIKE CRYSTALS IN PLASMA CELLS IN A PATIENT WITH A PLASMA CELL PROLIFERATIVE DISORDER. (Eng.) Stavem, P. (Section of Haematology, Medical Department A, Rikshospitalet, Oslo, Norway); Vandvik, B.; Skrede, S.; Hovig, T. *Scand. J. Haematol.* 14(1):24-34; 1975.

Needle-like crystals discovered in a large portion of plasma cells in a 57-yr-old man with a plasma cell proliferative disorder, were studied by light, immunofluorescent, and electron microscopy. During a 4-yr observation period, the number of plasma cells in the bone marrow remained unchanged (5-8%) as did their appearance. Approximately half of these cells contained several needle-shaped inclusions in their cytoplasm. These inclusions appeared unstained by May-Grunwald and Giemsa, PAS, Methyl Green, and Pyronine, but may have been dissolved or rendered unstainable during the fixation procedure. The crystals were not stained by immunofluorescent antisera to immunoglobulins. They were, however, observed only in cells staining for gamma heavy chains and kappa light chains. Ultrastructural observations revealed inclusions located centrally to the bulk of the rough endoplasmic reticulum and in close association with the Golgi apparatus. Inclusions were never found in the cisternae of the endoplasmic reticulum, but could be observed between the ergastoplasmic structures and occasionally near the plasma membrane. The inclusions were usually surrounded by a tri-laminar membrane. They exhibited parallel light and dark lines 30-40 A in diameter with a periodicity of 60-70 A at higher magnifications. Cytoplasmic filaments with a diameter of 90-100 A were sometimes found near the crystalline structures and elsewhere in the cytoplasm. The localization and structure of the inclusions suggests a protein nature. They are not, however, Russell bodies (which may have a crystalline structure but which are found within the cisternae of the endoplasmic reticulum). The occurrence of crystalline material may be related to the synthesis of monoclonal immunoglobulin proteins. Despite large and increasing Bence Jones proteinuria, the authors believe that the clinical course of the condition resembles benign monoclonal gammopathy rather than myelomatosis.

2219 STATIC AND FUNCTIONAL MORPHOLOGY OF THE PATHOLOGICAL PLATELETS IN PRIMARY MYELOFIBROSIS AND MYELOPROLIFERATIVE SYNDROME. (Eng.) Hattori, A. (Niigata Univ., Sch. Med., Japan); Koike, K.; Ito, S.; Matsuoka, M. *Ser. Haematol.* 8(1):126-150; 1975.

Native and functioning platelets in three patients with primary myelofibrosis and one with myeloproliferative syndrome were examined by transmission electron microscopy. Among various types of ultrastructural abnormalities in the platelets, three features were emphasized: hypoplasia of the surface connecting system (SCS) with few orifices, hyperplasia of the dense tubular system, and considerable variety in the numbers of granules. Morphological analysis was made on platelets of these patients and of normal subjects. Functional morphology of the abnormal platelets was examined in the aggregate samples either by ADP or collagen and the effluent blood from platelet retention test. The abnormal platelets were more or less indifferent from the aggregates and underwent only to a slight degree the changes observed in normal platelets. They frequently retained their native, round, smooth-surfaced form without pseudopod, and did not show the inward shift of organelles; furthermore, they retained more granules than did normal platelets. From these morphological findings, it is speculated that these ultrastructurally abnormal platelets had an impaired release reaction as a result of the dysfunction of SCS in passing released substances, of the inability of microfilaments to constrict in expelling these substances, and sometimes because of a lack of granules as the sources.

2220 THE ULTRASTRUCTURE OF THE PLATELETS IN REFRACTORY AMEMIA ('PRELEUKEMIA') AND MYELOMONOCYTIC LEUKEMIA. (Eng.) Maldonado, J. E. Mayo Clin., Rochester, Minn.). *Ser. Haematol.* 8(1):101-125, 1975.

Extensive morphological studies were conducted on the platelets of 16 patients with preleukemia or myelomonocytic leukemia. Although the degree and frequency of the changes varied in the different cases, it was evident that the platelets in these

wo pathologic states often were structurally abnormal. The abnormalities include changes in size (mainly giant forms), shape (frequent presence of round cells), and quantitative (particularly decreases) as well as qualitative changes in the platelet granules. Ovoid forms, balloon-shaped granules, granules with broad pointed extensions, and rod-like granules were present. A remarkable finding was the presence of giant granules of irregular contour and heterogeneous composition, perhaps the result of fusion of several single granules. Other changes included overabundance of the membranous systems of the platelet. The dense tubular system frequently complexed with the open canalicular system to form labyrinthine-like structures that occupied large areas of the cell surface.

221 'HAIRY' CELL LEUKAEMIA (LEUKAEMIC RETICULOENDOTHELIOSIS): A SCANNING ELECTRON MICROSCOPIC STUDY OF EIGHT CASES. (Eng.) Golomb, . M. (Univ. Chicago Hosp. Clin., Franklin McLean em. Res. Inst., Ill.); Braylan, R.; Polliack, A. r. *J. Haematol.* 29(3):455-460; 1975.

ells of eight patients with leukemic reticuloendoheliosis (LRE) were evaluated by scanning electron icroscopy and compared with chronic lymphocytic eukemia (CLL) cells. The surface architecture of he LRE cells generally resembled monocytes with road ruffles, undulating membranes and stub-like icrovilli which were confined to a limited area. hese surface features suggest that these characeristics are not cell-cycle, temperature-dependent r related to mode of preparation for scanning lectron microscopy. The LRE cells differed from LL cells by the presence of varying numbers of arrow microvilli. These observations suggest a ymphocyte-monocyte hybrid origin for the LRE cells, eaning toward the monocytic series. On the basis f surface features, scanning electron microscopy ay be a reliable method for the recognition of LRE ells.

222 CULTIVATION OF LEUKEMIC HUMAN BONE MARROW CELLS IN DIFFUSION CHAMBERS IMPLANTED INTO RMAL AND IRRADIATED MICE. (Eng.) Fauerholdt, L. igshospitalent, Univ. Hosp. Copenhagen, Denmark); acobsen, N. *Blood* 45(4):495-502; 1975.

e effect of environmental and cellular factors on e maturation defect in acute leukemia *in vivo* was udied using the diffusion chamber technique. Bone rrow was aspirated from the posterior iliac or ernum bones of eight untreated patients with acute eloid leukemia (AML). Eight 10-wk-old irradiated, male mice (strain NMRI) were used as chamber re- pients. After seven days of culturing, half of e chambers were retransplanted into irradiated ce; the remaining chambers were transplanted into unirradiated group. All were cultured for 19 ys. Chamber contents were then weighed, and sames were taken for differential cell counts. The ll number was higher in the irradiated group, but t to significant levels. Maturation consisted nongranulated and granulated blast cells. The myelocyte stage was not reached, but there was an absolute increase in promyelocytes in all cases. No difference was observed between cells in irradiated and normal mice. Cellular maturation in AML is apparently due to cellular, not environmental factors.

2223 INFECTIOUS MONONUCLEOSIS PRECEDING ACUTE MYELOMONOCYTIC LEUKEMIA. *(Eng.)* Pedersen, P. R. (Walter Reed General Hosp., Washington, D.C. 20012); Gerber, P.; Sweeney, G.; Blom, J. *Am. J. Med. Sci.* 269(1):131-135; 1975.

The case report of a 22-yr old white male with acute myelomonocytic leukemia associated with infectious mononucleosis is presented. Laboratory findings showed a hematocrit of 22%, a platelet count of 5,000/mm^3, and a WBC count of 9,000/mm^3. The peripheral smear showed a high percentage of blasts, most with 2-3 prominent nucleoli; 3% contained Auer rods. A bone marrow biopsy revealed a packed cellularity with depressed erythroid activity and 79% blasts. Buffy coat cells were cultured. For comparison, WBC from a normal male adult donor were also cultured. After 9-11 days of culture, most of the patient's cells consisted of large blastoid mononuclear cells, many of which were in mitosis. These cells continued to multiply. WBC cultured from the normal donors showed no morphologic or metabolic changes until the 53rd day. There was no growth in cultures of WBC from other patients with acute leukemia. In 1968, the patient had developed fever, splenomegaly, and minimal cervical adenopathy associated with a pharyngitis. A mono-spot screening test was positive and a diagnosis of infectious mononucleosis was made. In 1969, the patient had a recurrence of the same symptoms and a mono-spot screening test was positive. Abnormal heterophil studies done at the time also supported a diagnosis of infectious mononucleosis. This is the first case of granulocytic leukemia reported in which active infectious mononucleosis occurred simultaneously with the onset of acute leukemia. This is also the first case of this type in which the diagnosis of infectious mononucleosis was supported by lymphocyte culture data.

2224 ACUTE LYMPHOBLASTIC LEUKAEMIA: A HETEROGENOUS DISEASE. (Eng.) Haegert, D. G. (Addenbrooke's Hosp., Cambridge, England); Stuart, J.; Smith, J. L. *Br. Med. J.* 1(5953):312-314; 1975.

Eleven cases of acute lymphoblastic leukemia (ALL) were examined for four surface markers using a cytocentrifuge technique. In six cases, the neoplastic cells expressed B-lymphocyte surface markers. In four cases, less than 5% of the lymphoblasts formed sheep, Fc, or C3 rosettes and no evidence of surface immunoglobulin (Ig) was found. In one case, over 10% of the lymphoblasts formed sheep RBC and Fc rosettes, suggesting a T-cell origin for the blast population. In another case, significant numbers of Fc and C3 rosettes formed but the mixed antiglobin (MAG) test did not show significant numbers of cells with surface Ig. These findings suggest a B-lymphocyte

origin. Either the fluorescent antibody or MAG test demonstrated that a significant number of the blast cells of five patients had surface Ig. Comparison with controls indicated that the Ig was not a cytophilic antibody. Trypsin digestion of the lymphoblasts from one of these cases did not decrease the number of Ig-positive cells detected, suggesting a B-cell origin. Four of the five patients with detectable Ig on the surface of their lymphoblasts had been studied before treatment. Examination of the lymphoblasts of one of these patients suggests that they persist during the course of the disease. Apart from this, there was no correlation between the presence or absence of markers with the prognostic assessment at diagnosis, based on WBC count, percentage blasts, and presence or absence of mediastinal enlargement on chest X-ray picture. The data indicate that ALL is a disease of heterogenous origin divisible into four groups: those with T-cell markers, those with B-cell markers, those with both, and those with neither.

2225 ISOCHROMOSOME 17 IN A CASE OF EOSINOPHILIC LEUKAEMIA: AN ABNORMALITY COMMON TO EOSINOPHILIC AND NEUTROPHILIC CELLS. (Eng.) Mitelman, F. (Department of Internal Medicine, Lasarettet, S-22185 Lund, Sweden); Panani, A.; Brandt, L. *Scand. J. Haematol.* 14(4):308-312; 1975.

Chromosome analyses using the trypsin-Giemsa banding technique were performed on the bone marrow cells of a 50-yr-old man with eosinophilic leukemia to examine the relation between this disease and chronic myeloid leukemia (CML). On two occasions, 50 and 100 cells were counted and 25 and 35 metaphases were karyotyped in detail. All of the 150 cells counted had chromosome number 2n = 46. In all metaphases karyotyped, one chromosome 17 was replaced by a marker chromosome identified as an isochromosome for the long arm of chromosome 17. From a skin fibroblast culture, 50 cells were counted and 10 were karyotyped; all cells analyzed showed a normal male karyotype. No cell with Ph^1 chromosome was found in either analysis. The cytogenetic data suggest that eosinophilic leukemia is not a variant of CML and that both eosinophilic and neutrophilic cells are involved in the leukemic process.

2226 GENESIS OF THE PHILADELPHIA CHROMOSOME: POSSIBLE POINTS OF BREAKAGE IN CHROMOSOME NO. 22. *(Eng.)* Pravtcheva, D. (Centre of Oncology Sofia--Darvenitza, Bulgaria); Manolov, G. *Hereditas* 79(2):301-303; 1975.

Comparisons between the normal chromosome No. 9 and chromosome No. 22, on one side, and the 9q+ and the Ph^1, on the other were made in patients with chronic myelogenous leukemia (CML). Evaluation of size and appearance of the chromosome segments involved in the origin of these differences was attempted. WBC from peripheral blood of eight patients with CML were cultured and G-banded. In G-banding the Ph^1 chromosome appears as a small light m chromosome with only little difference between the short and the long arm. The centromeric region is usually stained and the long arm lacks the faintly stained band 22q12. In the normal No. 22, this band is located slightly proximal to the middle of the long arm. It was observed that the long arm of Ph^1 was decidedly longer than the light band 22q11. It is evident that the long arm of Ph^1 is approximately the same size as 22q13 or the same size as 22q11 plus q12. The author suggests that the genesis of the Ph^1 cannot have taken place simply by cutting off chromosome No. 22 at a location proximal to q12. Alternative possibilities are suggested: (1) A reciprocal translocation exists between No. 9 and No. 22 with the breaking points in the light band 9q34 and at the boundary between 22q11 and q12. The Ph^1 would thus include 22q11 and the terminal light segment of 9q34, (2) An insertion exists of 22q12 into 9q34. According to this alternative the Ph^1 would include 22q11 and q13. Both mechanisms would leave the changed long arms with normal telomeres, and the chromosomes in question would be expected to have full mitotic stability. In the first suggestion, only two breaks are required; in the second possibility, three are required: one in No. 9 and two in No. 22.

2227 ADDITIONAL CHROMOSOMAL INDICATION FOR THE UNICELLULAR ORIGIN OF CHRONIC MYELOCYTIC LEUKEMIA. (Eng.) Hossfeld, D. K. (Innere Universitatsklinik und Poliklinik der GHS Essen, D-4300 Essen 1, Hugelandstrasse 55, West Germany). *Z. Krebsforsch.* 83(4):269-273; 1975.

Chromosome analysis of bone marrow preparations from a 33-yr-old woman with chronic myelocytic leukemia (CML) was undertaken. Of 108 metaphases analyzed, the Philadelphia chromosome occurred in 100% and suggested a supernumerary chromosome 4-5 and a missing chromosome 6-12. In addition, 6 of the 108 metaphases had two Philadelphia chromosomes and a missing chromosome 19-20. By means of G-banding it was found that deleted material from chromosome 22 (the Philadelphia chromosome) was translocated to a variant chromosome 9 with an unusually long secondary constriction. Although the patient also had a variant chromosome 1, the translocation 9q+; 22q- was restricted to the variant chromosome 9. The variant chromosome 1 was transmitted to the patient by the mother, and the variant chromosome 9 was transmitted by the father. The translocation observed here supports previous evidence for a single cell origin of CML.

2228 CHROMOSOMES AND CAUSATION OF HUMAN CANCER AND LEUKEMIA. XVI. BANDING STUDIES OF CHRONIC MYELOCYTIC LEUKEMIA, INCLUDING FIVE UNUSUAL Ph^1 TRANSLOCATIONS. (Eng.) Hayata, I. (Roswell Park Memorial Inst., 666 Elm St., Buffalo, N.Y. 14263); Sakurai, M.; Kakati, S.; Sandberg, A. A. *Cancer* 36(4):1177-1191; 1975.

Forty-two Ph^1-positive cases of chronic myelocytic leukemia (CML) were examined with chromosomal banding techniques. Thirty-seven of these cases had Ph^1 translocation between chromosomes 9 and 22 [t-(9;22)(q34;q11)] in the Ph^1-positive marrow cells;

five cases had unusual types of Ph[1] translocation. Of the 37 cases, 21 had additional numerical and/or structural chromosomal changes, two had a missing Y chromosome, and one had an extra Ph[1] in the Ph[1]-positive cells. In the five cases with unusual types of Ph[1] translocation, chromosomes 2, 9, 10, and 13 were involved. The clinical picture in these five patients did not differ materially from that of the other Ph[1]-positive patients with CML, probably indicating that the recipient chromosome, with which the translocation from 22 takes place, does not play a crucial role in the course of the CML. In the 21 cases with abnormal karyotypes, nonrandom chromosomal changes were observed. Most of the changes were related to events occurring at the centromeric region. The prognosis of cases with only an extra 8 or Ph[1] appears to be better than that for cases with an iso-17q [i(17q)] chromosome or other extra chromosomes. The presence of the Ph[1] chromosome indicates the diagnosis of Ph[1]-positive CML. In some cases, however, the karyotypic events may be more complicated than just the deletion of chromosome 22, associated with the translocation to chromosome 9.

2229 TRISOMY 8 IN ACUTE MYELOID LEUKAEMIA. (Eng.) Philip, P. (Dept. of Medicine A, Rigshospitalet, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark). *Scand. J. Haematol.* 14(2):140-147; 1975.

A banding technique was used to identify a chromosomal abnormality, trisomy 8, in the bone marrow cells of two cases of acute myeloblastic leukemia. Twenty-five mitoses prepared before therapy from the bone marrow of a 15-yr-old boy were studied, and all were found to carry an extra chromosome 8 (47, XY, +8). Ten mitoses from a phytohemagglutinin (PHA)-stimulated blood culture were all normal (46, XY). Fifty mitoses from bone marrow therapy in a 44-yr-old woman all contained an extra group C chromosome. In the ten mitoses banded, the extra chromosome was a No.8 (47, XX, +8). These results confirm those of a single previously published case of trisomy 8 in bone marrow cells. The literature on myeloproliferative disorders with chromosomal abnormalities is reviewed. It is noted that among group C anomalies in the acute myeloid leukemias, only the involvement of chromosomes 7, 8, and 9 have been reported. Trisomy C has, however, been associated with both malignant and nonmalignant myeloid disorders, and is not restricted to acute leukemia.

2230 CYTOGENETIC ANALYSIS OF X-RAY-INDUCED CHROMOSOME ABERRATIONS IN SPONTANEOUSLY LEUKAEMIC AKR MICE. (Eng.) Szollar, J. (Univ. Res. Team of Medical Radiology, Budapest VIII, Hungary). *Mutat. Res.* 29(3):423-432; 1975.

Comparative cytogenetic and hematological examinations of AKR and CBA/H-T_6T_6 (healthy controls) mice were conducted to investigate chromosomal fragility in the two strains. Changes in the frequency of numerical and structural chromosomal aberrations in response to acute whole-body irradiation (100-600 R) were studied *in vivo*. The numbers of WBC decreased in both strains with increasing x-ray doses; no significant differences were observed between CBA and AKR mice. No leukemic sign was found in AKR mice, either in the number of WBC or in the qualitative smears of the peripheral blood and bone marrow after 48 hr x-irradiation. The mitotic index also showed a dose-dependent decrease without any significant difference between the strains. The percentage of the total number of aneuploid cells showed a significant difference between the dose-dependent increasing curves of CBA and AKR mice for irradiation doses greater than 100 R; this difference was primarily due to the frequency of hypoploid chromosome aberrations. Structural chromosome aberration frequency increased with the dose and at each dose level; the frequency was greater in AKR cells. The distribution of metacentric chromosomes correlated well with the increasing number of hypoploid cells in AKR mice. The number of induced rings did not increase as rapidly as the metacentrics with increasing dose; again, AKR cells showed a significantly greater percentage of rings than CBA cells for all dose levels tested. The increased chromosomal fragility of AKR bone marrow cells 5-7 mo before the manifestations of leukemia may be important for the development of malignant conditions by creating a weaker system that is more sensitive in carcinogenic agents.

2231 GENETIC DETERMINANTS OF MORPHOLOGICAL DIFFERENTIATION IN A LYMPHOMA-SARCOMA HYBRID. (Eng.) Cochran, A. J. (Dept. Pathol., Univ. Glasgow, Scotland); Wiener, F.; Klein, G.; Harris, H. *J. Pathol.* 115(1):1-12; 1975.

Variations in the histology and cytology of tumors growing *in vivo* in a syngenic population of mice innoculated with a methylcholanthrene-induced sarcoma/Moloney leukemia virus-induced murine lymphoma hybrid grown *in vitro* were studied; the relationship of these variations to alterations in the chromosome complement of the cells was examined. Cell fusion was used to produce the hybrid. After fusion the modal chromosome number was the sum of the two parental modes (69), with a narrow range of single-armed and biarmed chromosomes. During the 2-yr cultivation *in vitro* the modal chromosome number fell, while the range of total chromosomes and of biarmed chromosomes increased. Analysis of cells *in vitro* and of 55 *in vivo* tumors revealed that the reduction predominately affected the single-armed chromosomes: biarmed chromosomes proved to be extremely stable. Histological examination of 70 tumors revealed 21 classified as sarcoma-like, ten as lymphoma-like, and 39 as intermediate (of which 22 fell into a subclass described as cytologically intermediate and 17 were classed as histologically intermediate). A comparison of histological and karyological analyses for 39 tumors showed that an unreduced chromosome pattern was associated with sarcoma-like tumors (8 of 11 cases); one histologically intermediate tumor showed an unreduced chromosome pattern. Nineteen of 24 intermediate tumors were associated with a reduced pattern, as were 3 of 4 lymphoma-like tumors and two sarcoma-like tumors. The remaining tumors had a mixed population of cells. It appears that the genes controlling sarcoma-like differentiation are located on single-armed chromosomes because the loss

of sarcomatoid differentiation is associated with the loss of chromosomes. Lymphomatoid differentiation appears to be unaffected by chromosome loss; only loss of a fairly large number of chromosomes permits reappearance of lymphoma-like cells.

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The case report of a 19-yr-old female with short stature (148 cm), gonadal dysgenesis, and bilateral gonadoblastoma was presented to illustrate the slight phenotypic differences between patients with Y_p and Y_q dicentric chromosome. There were three cell lines in the cultivation of peripheral blood: the major cell line 46,XY dicentric in 43% of the cells; the 45,X line in 37%; and a minor line, 46,XY_q in 20% with a small Y chromosome in which the distal part of the long arm was deleted. In the skin the majority of the cells had a 45,X complement (25 cells); the 46,XY dicentric was found in five cells, and the 46,XY_q complement was found in one cell. The chromosomal material of the dicentric Y_p and Y_q prevented Turner's malformations and induced some rudimentary gonadal development. This case supports the probable localization of the male-determining loci and genes controlling the somatic development in the Y_p and the proximal part of the Y_q.

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See also:

* (Rev): 1809, 1810, 1811, 1822, 1839, 1840, 1843, 1844, 1847
* (Chem): 1857, 1868, 1873, 1891, 1905, 1912, 1915, 1925, 1962, 1969
* (Phys): 1984, 1988
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During 1960-1969, 321 reported deaths among Alaskan natives (Eskimos, Indians, and Aleuts) were attributed to cancer. This number is not significantly different from the cancer mortality of United States (US) Caucasians during this period, but it is significantly higher than that of US Indians. Age-specific mortality rates for all cancers combined were generally higher among Alaskan natives than Alaskan Caucasians in both men and women. Compared with US Caucasians, the rates among the natives were higher during a 20-yr span beginning at age 30 in women and age 35 in men. Beyond age 55, the rates dropped below US rates in men, but remained higher in women. Despite the few deaths reported each year, cancer mortality increased significantly in native males from 1960-1969. A decrease in mortality in native females was not significant. There was a ten-fold excess of nasopharyngeal cancer (NPC) in male and female Alaskan natives compared with US Caucasians and Indians. Five of the eight male NPC deaths, but neither of the two female deaths, were among Aleuts. Mortality was also high for cancers of the kidneys, esophagus, and salivary glands in Alaskan natives of both sexes. Three of the six female kidney cancer deaths were in Aleuts, but no excess was observed in male Aleuts. Eskimos accounted for six of the seven male deaths and two of the four female deaths from cancer of the esophagus. The three salivary gland cancer deaths were in two Eskimos and one Indian. Among Alaskan Caucasians, only NPC was in excess in both sexes. Deficits in mortality among Alaskan Caucasians for cancers of other sites may be attributable in part to selection factors associated with the migration of healthy workers into the state.

2298 MULTIPLE PRIMARY NEOPLASMS IN BLACKS COMPARED TO WHITES. IV. FURTHER CANCERS IN PATIENTS WITH CANCER OF THE DIGESTIVE ORGANS. (Eng.) Newell, G. R. (Tulane Med. Cent., New Orleans, La.); Krementz, E. T.; Roberts, J. D. *J. Natl. Cancer Inst.* 54(2):331-334; 1975.

The occurrence of second cancers in patients with initial cancer of the esophagus, stomach, pancreas, large intestine, and anorectal area was studied in all patients entering the Charity Hospital Tumor Registry from 1948-1978. Observed second primary cancers were compared to expected numbers to obtain a direct estimate of risk. The ratio of observed to expected cancers on the basis of person-year experience is equivalent to a relative risk (RR). In both Negro and Caucasian women, large excesses of invasive cancer of the cervix (RR=7.5) and corpus uteri (RR=2.9) were found after an initial cancer of the large intestine. No excess of breast cancer was found. In Negro women, the RR was 3.3 after a primary cancer of the pancreas, 1.6 after a large intestine primary, and 2.9 after an anorectum primary. The data suggest that both Negro and Caucasian men carry about a 2-fold excess risk of subsequent cancers, especially at sites in the digestive system, after a primary cancer in one of the above sites. In Caucasian men, a statistically significant increased risk was limited to patients with an initial cancer of the stomach (RR=2.1) and anorectum (RR=1.9). The finding of increased risk of subsequent skin cancer among white men with stomach cancer is considered an artifact caused by poor registration of skin cancer. Knowledge of the increase in risk for second primary cancers is important for physicians; these high-risk patients should be closely monitored.

2299 CANCER OF THE LUNG IN MALES. (Eng.) Jimenez, F. (Brooklyn Veterans Adm. Hosp., N.Y.); Teng, P.; Rosenblatt, M. B. *Bull. N.Y. Acad. Med.* 51(3):432-438; 1975.

A ten-year autopsy study of 465 cases of lung cancer in males at the Brooklyn Veterans Administration Hospital revealed the incidence to be 46.3% of total carcinomas and 16.4% of total autopsies. There was no trend toward escalation of the disease during this period. In 152 cases, the diagnosis was established by partial autopsy or by needle biopsy, leaving 313 cases with sufficient histologic material to permit complete microscopic examination. In 253 cases (80.8%) the diagnosis of bronchogenic carcinoma was confirmed; in 60 cases (19.2%) the diagnosis was considered questionable. The 253 cases in which the diagnosis was confirmed histologically represented 54.4% of the 465 cases originally certified. Among the 60 cases erroneously diagnosed there were 14 with no evidence of malignancy and 46 in which pulmonary metastases from extrapulmonary sites simulated bronchogenic carcinoma. The extrapulmonary sites included a variety of organs, most often the pancreas and colon. Among the 253 confirmed cases of lung cancer, the right upper lobe was the most frequent site of origin (40% of the cases) followed by the left upper lobe (36.0%). There were 110 (43.5%) cases of well-differentiated squamous cell carcinoma, 106 (41.9%) cases of undifferentiated carcinoma including oat-cell tumors, and 37 (14.6%) cases of adenocarcinoma including bronchiolo-alveolar types. The vast majority of cases showed multiple metastatic lesions.

2300 INCIDENCE OF PROSTATIC CARCINOMA IN THE ELDERLY. (Eng.) Rullis, I. (Veterans Admin. Hosp., Syracuse, N. Y. 13210); Shaeffer, J. A.; Lilien, O. M. *Urology* 6(3):295-297; 1975.

To obtain statistically valid data on the incidence of prostatic cancer in the elderly, specimens from 57 consecutive autopsies on men 80-yr-old and above were studied. Of 51 specimens from the 80-89 yr age-group, 34 were histologically malignant (carcinoma, incidence 66.7%). Of the 6 specimens from men over ninety, 5 contained carcinoma. The average wt of prostates containing carcinoma was 40 g compared to 28 g for those free of cancer.

2301 PATTERN OF NEOPLASTIC DISEASE IN CHILDREN WITH SPECIAL REFERENCE TO MALIGNANT TUMOURS. (Eng.) Pathak, I. C. (Postgraduate Inst. Medical Education and Res., Chandigarh., India); Datta, B. N.; Aikat, B. K.; Reddy, M. M.; Bhattacharjee, N. *Indian J. Cancer* 12(1):46-55; 1975.

Of 636 tumors seen in Indian infants and children (up to age 14 yr) from July 1964 to December 1972, 280 were malignant. Fifty-nine percent of the malignant tumors were mesenchymal tumors; tumors of hemopoietic tissue (72 cases) and leukemia (23) constituted 57.5% of all mesenchymal tumors. The male/female ratio for the malignant hemopoietic tumors was 6:1. Non-Hodgkin's lymphomas were much more common than Hodgkin's disease. The most common histologic type was lymphocytic lymphoma (27 cases), which had an average age incidence of 6.6 yr. Malignant tumors of soft tissue (45 cases) and of the skeletal system (25 cases) were the next most frequent types of malignant tumors. Embryonal tumors constituted only 18.2% of the malignant tumors, with nephroblastomas and retinoblastomas predominant. Gliomas of the CNS accounted for 12% of all the malignancies. The male/female ratio of the gliomas was 5:1 and the average age was seven years. There were only ten cases of malignant epithelial tumors, of which the most common were squamous carcinoma of the palate and larynx. Only eight of 22 teratomas were malignant. Nearly 60% of the 356 benign tumors also arose in mesenchymal tissue, and 128 (38.8%) of the benign mesenchymal tumors were hamartia. The relative incidence of leukemia, glioma, and lymphoma in Indian children is intermediate between that found in Western countries and in tropical Africa.

2302 THYROID NODULARITY IN CHILDREN. (Eng.) Rallison, M. L. (Univ. Utah Medical Center, Salt Lake City, Utah 84132); Dobyns, B. M.; Keating, F. R., Jr.; Rall, J. E.; Tyler, F. H. *Jama* 233(10):1069-1072; 1975.

A total of 5,179 school children (aged 11-18 yr) were surveyed in Utah, Nevada, and Arizona for thyroid abnormalities because of possible exposure to radiation from fallout. Nodularity of the thyroid was found in 93 children (1.8%). In 34, the nodularity represented lobulation associated with adolescent goiter, and in 31, thyroiditis. Two malignant neoplasms were found. In a normal childhood population in which nodularity is incidentally discovered on physical examination, the risk of nodularity being malignant is approximately 2%. Factors that favor exploration of thyroid nodules in children are discreteness, growth of the mass, singleness, and absence of other thyroid disease.

2303 A RETROSPECTIVE STUDY OF THE CANCER PATTERNS AMONG HOSPITAL IN-PATIENTS IN BOTSWANA 1960-72. (Eng.) Macrae, S. M. (Dept. Social Medicine, Univ. Birmingham, B15 2TJ Birmingham, England); Cook, B. V. *Br. J. Cancer* 32(1):121-133; 1975.

Records of approximately 310,000 patients admitted to the ten hospitals in Botswana between 1960 and 1972 were studied, and details of 1,445 patients with malignant tumors were abstracted. For the 894 tumors for which there was some supporting evidence (at best histological proof, and minimally a clinical description of symptoms) proportional frequencies were calculated for all sites, and comparisons were made with the findings of other surveys. Cancer of the cervix uteri was found to be overwhelmingly the most commonly occurring malignant tumor, and the proportional frequency (39.8%) was among the highest observed in Africa south of the Sahara. Skin tumors were unusually common for Southern Africa in both sexes (15.1% in males, 8.7% in females). In men, penile and prostatic tumors had a relatively high frequency (4.3% and 9.0%, respectively), while the frequency for liver was lower (8.7%) than in other parts of Southern Africa; the frequency for lung was also lower. Esophageal cancer in men had a moderate frequency (10.7%). Other tumors that showed a marked variation of frequency within Africa (Kaposi's sarcoma and cancers of the stomach and bladder) were all low in frequency in Botswana. Tumors that were rare throughout Africa but common in Western Europe and North America (cancers of the colon, rectum, and corpus uteri) were also rare in Botswana.

2304 CANCER INCIDENCE RATES IN THE DALLAS-FORT WORTH METROPOLITAN AREA. (Eng.) Frenkel, E. P. (Univ. Texas Southwest. Med. Sch., Dallas); Clark, B.; Percy, C. *Tex. Med.* 71(3):71-78; 1975.

Data on the histologic types of cancer and demographic information of cancer patients in the Dallas-Fort Worth area from 1969 to 1971 were obtained. This data and previous studies done in the same area in 1938 and 1948 were compared. The Dallas-Fort Worth area was also compared with eight other geographic areas in the United States. The nine areas represent a data base in excess of 10% of the U. S. population. Data was provided by medical facilities from the communities. Rates were expressed per 100,000 population using 1970 census figures. The incidence rate of primary invasive cancer is the average annual number of cancers divided by the population base. Cancer of the lung was higher in the Dallas-Fort Worth area than in all other areas, and exceeded the incidence for colon-rectum cancer (the most common form in the total areas of the survey). Cancers of the buccal cavity and melanoma were considerably higher than for the rest of the survey area. Cancers of prostate and pancreas were essentially the same as other areas, while urinary bladder and stomach sites had lower rates than in the other areas. Cancer of the breast in white women was lower in the Dallas-Fort Worth area than the rest of the survey areas; the incidence in black women was significantly less than that in white women in the overall study. Overall changing trends show climbing rates for men, declining rates for women, a 50% decline in stomach cancer in both sexes, a decline in uterine cancer, but a 10-fold increase in lung cancer.

2305 HEPATITIS B SURFACE ANTIGEN AND LIVER CELL CARCINOMA. *(Eng.)* Williams, A. O. (Dept. Pathology, Univ. Ibadan, Nigeria). *Am. J. Med. Sci.* 270(1):53-56; 1975.

The possible relationship between hepatitis B antigen (HB_s Ag) and liver cell carcinoma was examined. The frequency distribution of HB_s Ag in different parts of the world reveals a relatively high frequency among healthy members of population groups inhabiting areas of high incidence of liver cell carcinoma. Similar high frequencies of HB_s Ag are also found in those areas where macronodular cirrhosis is relatively common and is usually complicated by liver cell carcinoma. In geographic areas with low incidence of liver cell carcinoma and macronodular cirrhosis, a relatively low frequency of HB_s Ag is usually encountered in the population. The frequency of HB_s Ag is relatively higher in patients with liver cell carcinoma with or without cirrhosis than in comparable controls. The subtypes of the antigen do not correlate with the incidence of liver cell carcinoma and there is also no correlation between alpha fetoprotein and HB_s Ag in the presence of liver cell carcinoma. HB_s Ag is very rarely detected in patients with micronodular cirrhosis or in liver cell carcinoma which may be its complication. It would appear that HB_s Ag is necrogenic in the liver and is capable of producing hepatic necroses or hepatitis which may progress to macronodular cirrhosis. The areas of hepatic necroses may either progress to liver cell carcinoma or the resultant macronodular cirrhosis may be complicated by carcinoma. The oncogenic potential of HB_s Ag requires further studies.

2306 UNEXPECTED HIGH INCIDENCE OF PRIMARY LIVER CANCER IN GENEVA, SWITZERLAND. (Eng.) Tuyns, A. J. (Int. Agency Res. Cancer, Lyon, France); Obradovic, M. *J. Natl. Cancer Inst.* 54(1):61-64; 1975.

A newly established cancer registry in Geneva recorded an age-standardized incidence rate of primary liver cancer of 9.7/100,000 for males, a figure 4-5 times higher than rates reported elsewhere in Europe. A search was made for a bias in coding, reporting, or diagnosing to determine whether the incidence of primary liver cancer was genuinely high in Geneva. The mode of diagnosis was ascertained, and the original reports were checked. During 1970-1972, 60 male and 11 female residents of Geneva were reported to the registry as having primary liver cancer. Sixty-one (86%) cases were confirmed by autopsy and eight (11%) by biopsy. For two patients, only a death certificate was available. Cirrhosis was present in 40 (56.3%) cases, absent in 20 (28.2%) and not mentioned in the reports for 11 (15.5%) patients. Hepatitis was recorded on only two patients. Thirty-three patients (46.5%) were alcoholics. Most lesions were pure hepatocarcinomas (53 cases, 74.6%) or mixed cholangiohepatocarcinomas (eight cases, 11.3%). It is concluded that the high incidence of primary liver cancer in Geneva is genuinely high. Whether the much lower incidence in the European countries with cancer registration is due to some underestimation is not certain. The high proportion of associated cirrhosis and alcoholism suggests a possible etiologic relationship.

2307 THE EPIDEMIOLOGY AND ETIOLOGY OF ESOPHAGEAL CANCER IN NORTH CHINA: A PRELIMINARY REPORT. (Eng.) Coordinating Group for Res. on Etiology of Esophageal Cancer in North China. *Chin. Med. J.* 1(3):167-183; 1975.

The mortality rates from esophageal cancer in three North China provinces (Honan, Hopei, and Shansi) and Peking were surveyed for the period 1969-1971. In addition, an epidemiologic survey of esophageal cancers among domestic animals was made, and animal experiments were performed to study the etiology and prophylaxis of esophageal cancers. The average age- and sex-adjusted mortality rate from esophageal cancer in the study area was 37.39/100,000; the rate varied from 139.80/100,000 in Hehpih County to 1.43/100,000 in Hunyuan County. The mortality rates from esophageal cancer in Linhsein County had remained stable during the period 1941-1970. The male:female ratio averaged 1.6:1.0 and tended to decrease with increasing overall mortality rate. The incidence of esophageal cancer increased with age in those over 30 yr old; over 60% of the total cases were aged 50-69 yr. The mortality rates among younger age groups increased as the overall mortality rate increased. In areas where high rates of esophageal cancer prevailed, there was also a high incidence of epithelial hyperplasia. The stages in the evolution of esophageal cancer could be divided into two groups: an early clinical group in which the only symptoms were a slight localized rigidity or mottling of the rugae, and a clinical group which showed classical symptoms. The ratio of esophageal cancer to cancer of the cardiac end was 3:1. In Linhsien and Fanhsien Counties, there was a correlation between the occurrence of pharyngeal and esophageal cancers in domestic fowl and man; correlations were also found between the amounts of secondary amines, nitrates, and nitrites in the food and the mortality rate from esophageal cancer. In addition, the amounts of various elements in the food and water (e.g., cobalt, nickel, and molybdenum) were lower in areas with high mortality rates. The fungus, *Geotrichum candidum* Link was found in the food of Linhsien County and was shown to be pathogenic in mice and a promoting factor to the carcinogenic properties of nitrosamines. Vitamin C administration and vitamin A deficiency reduced the carcinogenic properties of the nitrosamines in animals. The relation between epithelial hyperplasia and malignant change of the esophageal mucosa was also studied in rats, and facilities for clinical and laboratory field work were set up in high incidence areas.

2308 SQUAMOUS PAPILLOMA OF THE CERVIX UTERI: A TWO YEAR PROSPECTIVE CYTOLOGICAL STUDY. (Eng.) McKenna, H. (Royal Women's Hosp., Herston Road, Brisbane, Q. 4029 Australia); Fraser, M.; Silverstone, H. *Med. J. Aust.* 2(8):304-306; 1975.

A two-yr prospective study of the incidence, dif-

erentiated diagnosis, associated conditions, and havior of squamous papilloma of the cervix uteri s done at the Queensland Cytology Service. The ses were initially selected from those with a inical history of cervical warts. Squamous papil-ma was found in cervical smears from 27 patients 6-35 yr old) out of 147,813 consecutive examina-ons. Of the 27 with papilloma, 17 were pregnant. one of these cases the lesion progressed cyto-gically from mild to moderate squamous dysplasia. o patients were cured by biopsy, 13 lesions re-essed to lesser severity or normal, and 11 were t followed. Trichomonad infection was associated <0.02) with papilloma; fungal infections were as-ciated with the control group (P<0.01). The le-on that progressed may have been a true (malig-nt) papilloma; the others probably were cockscomb lyps or condylomata acuminata. This study sug-sts that neither cytomorphology nor histopathology liably indicate the biological behavior of the uamous papilloma.

309 RETINOBLASTOMA: AN EPIDEMIOLOGICAL STUDY (SURVEY AND REVIEW). (Eng.) Pawlak, B. . (Nat. Sci. Dep., Roswell Park Mem. Inst., Buffalo, .Y.). *J. Surg. Oncol.* 7(1):45-55; 1975.

review of factors involved in retinoblastoma is resented, and an epidemiological study of patients rom two cities was conducted. Retinoblastoma con-ists of highly undifferentiated cells that arise rom the neuroectodermal layer of embryonic tissue. t ranks fourth in sensitivity to radiation and oc-urs either as a hereditary or nonhereditary disease. ereditary retinoblastoma is characterized by auto-omal dominant inheritance, early age of onset, and ultiple tumors. Both sexes are affected equally. onhereditary retinoblastoma occurs sporadically, at later age, and with the development of a single umor. Its etiology is unknown. All bilateral ca-es are hereditary; most unilateral cases are con-idered nonhereditary. Unilateral retinoblastoma ccurs twice as often as bilateral retinoblastoma. resent chromosome analyses have not demonstrated n obvious chromosome abnormality distinguishing ereditary from nonhereditary retinoblastoma. In nilateral cases the affected eye is enucleated. In ilateral cases the more severely affected eye is nucleated. A more conservative method is to use adiation and chemotherapy (triethylenemelamine is he chemotherapeutic agent used most frequently). wenty-six cases were diagnosed between 1935 and 972 at hospitals in Buffalo, N. Y. One hundred nd forty-two cases were reported in Rio de Janeiro, razil between 1956 and 1973. Although the cases o not represent the total retinoblastoma population iagnosed in either city during the periods, retino-lastoma apparently occurs more frequently in Rio e Janeiro than in Buffalo. In Buffalo, the age at iagnosis for bilateral cases was 9.2 mo, compared ith 27.9 mo for the unilateral cases. There was o apparent difference in Rio de Janeiro. All of he Buffalo patients were treated radically. Only small population of the retinoblastomas were hered-tary there, as opposed to 81.3% of the Rio de Ja-eiro patients. The methods of treatment did not iffer significantly except for the use of orbital exenteration in treating unilateral patients in Rio de Janeiro. Over 50% of the Buffalo patients survived for at least five years. Only about 25% of the Rio de Janeiro patients survived for the same length of time. Patients in Rio were not diagnosed until they were 30-mo-old and the disease had progressed.

2310 CANINE THYROID NEOPLASMS: EPIDEMIOLOGIC FEATURES. (Eng.) Hayes, H. M., Jr. (Natl. Cancer Inst., Bethesda, Md. 20014); Fraumeni, J. F., Jr. *J. Natl. Cancer Inst.* 55(4):931-934; 1975.

A retrospective study of medical records from twelve veterinary hospitals yielded 144 dogs with a confirmed diagnosis of a thyroid neoplasm (25 adenomas and 119 carcinomas). Forty-five of these dogs had additional primary neoplasms. As in man, associated tumors suggested the inherited multiple endocrine adenomatosis, type 1, and a possible svndrome of thyroid and chemoreceptor lesions. Although the female preponderance of human thyroid cancer was not seen in dogs, fêmales showed a much sharper increase in risk with advancing age than did males. Three breeds (beagle, boxer, and golden retriever) had a significantly greater risk for thyroid carcinoma than did all dogs combined, whereas minature and toy poodles had a low risk. The function of thyroiditis in the origin of thyroid cancer, as suggested by reports of thyroid carcinoma in people with Hashimoto's disease, may be clarified by follow-up studies of beagles, which are prone to Hashimoto-type thyroiditis.

2311 AN EPIZOOTIC IN HAMSTERS OF LYMPHOMAS OF UNDETERMINED ORIGIN AND MODE OF TRANSMISSION. (Eng.) Ambrose, K. R. (Dept. Microbiol., Univ. Tennessee, Knoxville); Coggin, J. H., Jr. *J. Natl. Cancer Inst.* 54(4):877-880; 1975.

A spontaneous lymphoma epizootic that occurred in inbred LSH and randombred LVG Syrian hamsters is discussed. Both strains had been bred and raised in one breeding and holding room until needed for experiments when they were moved to the experimental room. Of the population of animals housed in the experimental room and subsequently killed because of the epizootic, approximately half bore lymphomas that had no observable connection with previous experimental treatments or manipulation. The earliest palpable tumor observed was a cervical lymph node lymphoma in a 4-wk-old LSH female. Among hamsters 17-19 wk old, half the LSH and LVG strains had lymphomas. Hamsters born outside the colony were subsequently housed in the colony with the tumor-bearing animals. No known direct physical contacts occurred between the animals in the afflicted colony and the newly introduced animals, but nine weeks after introduction into the colony housing lymphoma-bearing hamsters, animals in all age groups demonstrated lymphomas with frequency and pathology similar to those observed previously during the spontaneous epidemic. Most lymphomas (45%) arose along the small intestine as single or multiple lesions. Other major sites of primary tumor development were the mesen-

teric lymph node (19%), liver (11%), kidneys (10%), cervical lymph node (6%), and spleen (6%). A second colony of LSH and LVG hamsters was begun after exterminating the original colony and disinfecting the animal facilities for one month. After six months in which no tumors were observed, lymphomas recurred; these were similar in pathology and incidence to those seen previously. After the second colony was exterminated, the facilities were completely scrubbed, sprayed, and fumigated by aerosol with virucides and pesticides on a continuous basis for one month, and a containment operation was set up. A third colony was then established and lymphomas recurred four weeks later. Two possible explanations for this lymphomas transmission are suggested: (a) activation of an endogenous virus by some as yet undetermined environmental carcinogen, immunosuppressant, or vaccine treatment; or (b) transfer of an exogenous virus by direct or indirect contact.

2312 CANCER MORTALITY IN U.S. COUNTIES WITH PLASTICS AND RELATED INDUSTRIES. (Eng.) Mason, T. J. (Epidemiology Branch, Natl. Cancer Inst., Landow Building, Bethesda, Md. 20014). *Environ. Health Perspect.* 11:79-84; 1975.

An investigation of site-specific cancer mortality was conducted to evaluate the risk of malignancy among residents of counties in the U.S. with possible environmental exposure to vinyl chloride. The 1963 Census of Manufacturers was used to identify counties with chemical establishments engaged primarily in the manufacture of plastics, synthetic resins, synthetic rubber, cellulosic man-made fibers, or synthetic organic fibers. Those counties in which a relatively large percentage of the population was employed in these establishments were studied. Site-specific cancer mortality rates were calculated for the white population in these counties and in control counties as well as in the total U.S. for the period 1950-1969. All rates were age-adjusted. The data revealed an excess of multiple myeloma among men associated with the manufacture of synthetic rubber and synthetic fibers; the excess was significant only for the period 1965-1969. A causal relationship between these manufacturing categories and multiple myeloma could not be established, however. Among males and females, an excess of lung cancer was associated with synthetic rubber manufacture. Mortality rates for cancers of the nasal cavity and accessory sinuses and bladder among men were excessive only in counties with synthetic rubber manufacture. Mortality rates decreased with time for counties with plastic and synthetic resin industries, and for cancer of the nasal cavity and accessory sinuses among men in counties with synthetic rubber industry. Lung cancer among men increased at a greater rate in control counties than in counties with synthetic rubber manufacture, but for all other sites the rate of increase was greater in the manufacturing counties than in the controls or in the U.S. as a whole.

2313 ENVIRONMENTAL CONCERNS BEYOND THE WORKPLACE. (Eng.) Schweitzer, G. E. (Off. Toxic Subst., Environ. Prot. Agency, Wash., D. C.). *Ann. N.Y. Acad. Sci.* 246:296-302; 1975.

Preliminary investigations were undertaken by the Environmental Protection Agency on vinyl chloride monomer (VCM) and polyvinyl chloride (PVC) activities, especially their migration beyond the manufacturing plants. A nationwide sampling program to determine VCM levels in ambient areas was initiated at ten PVC plants. The need for the proposed Toxic Substances Control Act which would require reporting of industrial data is emphasized. Over 200 million and 50 million pounds of VCM and PVC, respectively, are estimated to be discharged into the air, water, effluents and sludge annually. A materials loss of 6% in the PVC production process was reported. Leakage typically occurs during opening of polymerization kettles, transfer, drying and disposal of oversize polymer particles. Increased maintenance has reduced loss to 4% in some plants. Many compounding and fabrication plants are attempting to reduce the amount of monomer associated with the polymer following polymerization since monomer concentration ranges between 500-1,000 ppm and may reach 7,000 ppm. Epidemiological studies of populations near PVC plants and toxicological tests *in vivo* are necessary in assessing effects of VCM exposure on the nonworker population. *In vitro* experiments on the significance of impurities in VCM, synergistic effects due to exposure to other chemicals, and metabolic reactions induced by VCM are necessary. The persistance and migration of VCM in the environment should also be studied. Physical properties of PVC must be considered with regard to disposal. Landfill disposal presents problems because long-term PVC stability cannot be guaranteed and HCl is produced when it is incinerated.

2314 THE INCIDENCE OF RETINOBLASTOMA. (Eng.) Devesa, S. S. (Natl. Cancer Inst., Landow Bldg., Rm B506, 7910 Woodmont Ave., Bethesda, Md. 20014). *Am. J. Ophthalmol.* 80(2):263-265; 1975.

Data from the Third National Cancer Survey indicate that the annual incidence of retinoblastoma in the United States is 11.0 new cases/million children under 5 yr. In response to a reported excess mortality among blacks, rates were computed by race. The rate for white children was 10.8 cases/million children and 9.8 cases/million for black children. Among the total of 61 newly diagnosed cases reported among residents of the survey areas, the 50 unilateral cases occurred in 24 boys and 26 girls, whereas the 11 bilateral cases occurred in nine girls and only two boys. The reasons for a female preponderance among the bilateral cases remain unclear.

2315 RADIOLOGICAL PRACTICE IN HIROSHIMA AND NAGASAKI: TRENDS FROM 1964 TO 1970. (Eng.) Sawada, S. (Dep. Radiol., At. Bomb Casualty Comm., Hiroshima, Japan); Fujita, S.; Russell, W. J.; Takeshita, K. *Am. J. Public Health* 65(6):622-633; 1975.

The use of medical X-ray in Hiroshima and Nagasaki from 1964-1970 was assessed according to numbers of

lms consumed, and the radiographic, fluoroscopic
d photofluorographic examinations performed. Large
spitals, health centers and the Atomic Bomb Casu-
ty Commission (ABCC) as well as small hospitals
d clinics were investigated between 1964 and 1970.
arly totals of X-ray films used for radiography
d fluoroscopy, and the total numbers of radio-
aphic, fluoroscopic, chest and gastric photofluo-
graphic examinations and radiation treatments were
termined for each institution. Half of the data
urces were technical records; the remaining infor-
tion was from X-ray films, medical charts or in-
rmation from physicians. Mean values were calcu-
ted from the large and small institutions from
ta for each year. The final values were derived
multiplying the means by the actual numbers of
spitals and clinics having X-ray equipment. A
eady increase in all forms of diagnostic proce-
res since World War II was shown. The number of
rays taken increased during the 7-yr period. How-
er, a comparison of these statistics and those of
her cities in Japan (Hiroshima and Nagasaki) re-
als that those figures do not reflect greater num-
rs of X-ray examinations.

16 APPARENT TRANSMISSION OF HODGKIN'S DISEASE [letter to editor]. (Eng.) Vianna, N. J.
ew York State Dept. Health, Albany, N.Y.); Polan,
K. *N. Engl. J. Med.* 293(9):458; 1975.

17 CHANGING EPIDEMIOLOGY OF LUNG CANCER: INCREASING INCIDENCE IN WOMEN [abstract].
ng.) Beamis, J. F. (No affiliation given); Stein,
; Andrews, J. L., Jr. *Chest* 68(3):401-402; 1975.

18 CANCER OF THE GENITAL ORGANS OF WOMEN IN THE AREA OF MAKSIMIR GYNECOLOGICAL
SPENSARY 1959--1973. (Cro.) Baudoin, V. (Gyne-
logical Dispensary, Public Health Dept. Maksimir,
arcova 20, 41000 Zagreb, SFR Yugoslavia). *Libri*
col. 4(1):23-31; 1975.

19 AGE DISTRIBUTION OF THE CERVICAL CANCER CASES. (Jpn.) Sugimori, H. (Sch. Medi-
ne, Kyushu Univ., Fukuoka, Japan); Kashimura, M.;
shimura, Y.; Matsuyama, T.; Hamasaki, Y.; Nishi-
ra, A.; Taki, I. *Gan No Rinsho* 21(13):1189-1194;
75.

320 TUMORS OF CHILDHOOD IN IBADAN, NIGERIA. (Eng.) Williams, A. O. (Univ. Coll.
sp., Ibadan, Nigeria). *Cancer* 36(2):370-378;
75.

321 A STUDY OF REGIONAL VARIATIONS OF CANCER MORTALITY IN ONTARIO. (Eng.) Chapman,
.-A. W. (Univ. Waterloo, Ontario, Canada). *Diss.*
bstr. Int. B 35(10):4778; 1975.

2322 LEUKEMIA MORBIDITY IN EREVAN. (Armenian) Uzunian, L. Kh. (No affiliation given); Petrosian, Zh. S.; Stepanian, R. M. *Zh. Eksp. Klin. Med.* 15(2):106-109; 1975.

2323 AN EPIDEMIOLOGICAL STUDY OF OCCUPATIONAL BLADDER TUMOURS IN THE DYE INDUSTRY OF JAPAN. (Eng.) Tsuchiya, K. (Keio Univ. Sch. Med., Japan); Okubo, T.; Ishizu, S. *Br. J. Ind. Med.* 32(3): 203-209; 1975.

2324 ASBESTOS IN DULUTH WATER. (Eng.) Wells, A. H. (Miller-Dwan Hosp., Duluth, Minn.). *Minn. Med.* 58(6):458-459; 1975.

2325 EXPOSURE TO ASBESTOS IN THE USE OF CONSUMER SPACKLING, PATCHING, AND TAPING COMPOUNDS. (Eng.) Rohl, A. N. (Mount Sinai Sch. Medicine, City Univ. of New York, New York, N.Y. 10029); Langer, A. M.; Selikoff, I. J.; Nicholson, W. J. *Science* 189(4202):551-553; 1975.

2326 COUNTING ASBESTOS FIBERS BY THE MOST PROBABLE NUMBER METHOD. (Eng.) Reist, P. C. (Dept. Environ. Sci. Eng., Univ. North Carolina, Chapel Hill). *Am. Ind. Hyg. Assoc. J.* 36(5):379-384; 1975.

2327 INDIRECT STANDARDIZATION AND MULTIPLICATIVE MODELS FOR RATES, WITH REFERENCE TO THE AGE ADJUSTMENT OF CANCER INCIDENCE AND RELATIVE FREQUENCY DATA. (Eng.) Breslow, N. E. (Dept. Biostatistics SC-32, Univ. Washington, Seattle, Wash. 98195); Day, N. E. *J. Chronic Dis.* 28(5/6):289-303; 1975.

2328 ESTIMATION OF THE PROLIFERATIVE POOL AND THE MITOTIC CYCLE TIME BY THE ACCUMULATION CURVE OF H^3-THYMIDINE LABELED CELLS. (Rus.) Gushchin, V. A. (Inst. Roentgeno-Radiology U.S.S.R. Ministry Public Health, Leningrad, U.S.S.R.) *Tsitologiia* 17(5):552-556; 1975.

See also:

* (Rev): 1811, 1812, 1813, 1814, 1815, 1816, 1822, 1825, 1830, 1831, 1832, 1833, 1845, 1846, 1847, 1848, 1849
* (Chem): 1882, 1917, 1942, 1966, 1968, 1969, 1970, 1972
* (Viral): 2034
* (Immun): 2151
* (Path): 2207

2329 PROLIFERATION OF MOUSE ASCITES MAMMARY CARCINOMA MM2 CELLS IN THE PRIMARY CULTURE. (Eng.) Mori, T. (Inst. Medical Science, Univ. Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan); Takaoka, T.; Katsuta, H. *Jpn. J. Exp. Med.* 45(2):123-131; 1975.

Culture conditions suitable for active proliferation in primary culture of transplantable mouse ascites mammary carcinoma MM2 cells were investigated. The most active growth was obtained in the medium consisting of 5% inactivated fetal calf serum and 95% RPMI-1640. Addition of estriol (1 μg/ml) increased the number of cell nuclei/tube with both MM102 and MM2 cell cultures. The optimum innoculum size was 1×10^5-2×10^5 cells/ml. MM2 cells preserved their tumorigenicity for five months of cultivation.

2330 *IN VITRO* STUDIES ON HUMAN CERVICAL EPITHELIUM, BENIGN AND NEOPLASTIC. (Eng.) Wilbanks, G. D. (Rush-Presbyterian-St. Luke's Med. Cent., Chicago, Ill.). *Am. J. Obstet. Gynecol.* 121 (6):771-788; 1975.

Data are presented on the efforts towards the establishment of a cell line from the human cervix and methods for transforming normal cells *in vitro*. Material included cervical epithelial cells from 166 patients with normal cervices, 18 with dysplasia and 29 with carcinoma *in situ* (CIS). Cell cultures were observed for the rapidity of outgrowths from the explants, cell type and cell-to-cell interactions mitotic mechanisms, length of growth in culture, transferability, and number of passages. Media and cultures were tested for evidence of bacterial, viral or mycoplasmic activity. Altered morphology or growth characteristics of experimental cultures were compared with appropriate controls and with past experience of the growth of unaltered similar cells. Chromosome counts and karyotypes, both transmission and scanning electron microscopy, growth under soft agar and animal inoculation were techniques used. The effects of various chemical carcinogens and herpes simplex viruses were studied. Cells grown for self-limited periods had the same characteristics as when grown *in vivo*. Normal cells grew in monolayer and differentiated within 6-8 wk. Various attempts to transfer primary cultures were all unsuccessful. Contact inhibition was exhibited; well-developed desmosomes and tonofibrils and regular nuclei were observed. Actively growing cells had regular short microvilli on the surface. Neoplastic cells (cultures from areas of dysplasia and CIS) grew as individual cells and 30-50 passages were possible; cells did not differentiate. Prophase was more active than normal, and there was slowing of telophase. There were few desmosomes and rare tonofibrils, and an increase in the number and complexity of microvilli. In a 10-yr period, three spontaneous transformations occurred in normal cells after 3-4 wk, and were transferred for 11, 12, and 22 passages. They showed an abnormal chromosome marker clustered around 80. The addition of methylcholanthrene resulted in a transformed culture passaged 21 times, with characteristics of cells from patients with CIS.

2331 ESTABLISHMENT OF A CONTINUOUS TUMOR-CELL LINE (PANC-1) FROM A HUMAN CARCINOMA OF THE EXOCRINE PANCREAS. (Eng.) Lieber, M. (Natl. Cancer Inst., Bethesda, Md.); Mazzetta, J.; Nelson-Rees, W.; Kaplan, M.; Todaro, G. *Int. J. Cancer* 15(5):741-747; 1975.

An epithelioid cell line, PANC-1, was started from a human pancreatic carcinoma of ductal cell origin, and has been maintained in culture for over 2 yr. The line was cultured on Dulbecco's medium containing 10% fetal calf serum and antibiotics at 37 C. The cells were serially transferred with 0.1% trypsin. Neoplastic cells were identified by monolayer culturing and by scoring after two weeks in soft agar culture; cells (5×10^6 cells/mouse) were also injected s.c. into nude-athymic mice. Chromosomes were analyzed by Q and G banding. The cells also underwent assay for glucose-6-phosphate dehydrogenase (G6PD), supernatant reverse transcriptase, and radioimmunoassay for carcinoembryonic antigen (CEA) and for α-fetoprotein. The line was subcultured 42 times. At passage 30, the cell doubling time in log phase was about 52 hr, and G6PD activity was the slow mobility or B type. The malignant nature of the line was verified by the ready growth in monolayer and soft agar and by anaplastic growth in one of the injected mice. There was a modal chromosome number of 63 with three distinct marker chromosomes and a small ring chromosome. The supernatant reverse transcriptase activity was negative at both high and low passages and no activity could be induced by treatment with 5-bromodeoxyuridine (100 μg/ml, 24 hr) or 5-iododeoxyuridine (20 μg/ml, 48 hr); electron microscopy at passage 35 showed no viral particles. The cells or cell culture did not contain significant levels of CEA or α-fetoprotein. Success with PANC-1 may be due to the low level of differentiation of the original tumor tissue. Enzyme and chromosome studies indicate that the line is of human origin, and that it appears unique in relation to other human cell lines studied by quinacrine banding techniques. PANC-1 may be useful in studies of pancreatic adenocarcinoma of ductal origin.

2332 TUMOR ANGIOGENESIS: A POSSIBLE CONTROL POINT IN TUMOR GROWTH. (Eng.) Folkman, J. (Harvard Med. Sch., Boston, Mass.). *Ann. Intern. Med.* 82(1):96-100; 1975.

It is proposed that tumor neovascularization is caused by a humoral stimulus secreted by the tumor; and that the capillaries which result are an important control mechanism in the growth of the tumor. Cells in a tumor live only because the tumor can stimulate the formation of new capillaries. The life of the tumor can be divided into the avascular phase, when the tumor is microscopic; and the vascular phase, when a humoral stimulus is secreted by the tumor to elicit new capillaries from the host. Skin metastases from an infant with rhabdomyosarcoma showed no capillaries if the nodules were 1.5 mm or less; larger skin metastases were vascularized. Once capillaries penetrate the tumor, rapid growth begins in 24 hr. Maximum tumor growth appears close

) a capillary unit. After tumor growth occurs, the lood supply is compressed by necrosis deep in the umor and new vascularization takes place. Tumor ngiogenesis can be seen in the absence of inflama-ion, an immune reaction, or a healing wound. The uthors have separated a diffusable factor (tumor ngiogenesis factor). When a disc containing the actor is placed on a chick chorioallantoic membrane, ew vessels converge on the disc in 48 hr. The most alignant neoplasm cannot reach maximum growth until ew capillaries are formed. Prolonging the avascu-ar phase is a suggested approach to therapy.

333 GENE EXPRESSION IN MOUSE NEUROBLASTOMA CELLS: PROPERTIES OF THE GENOME. (Eng.) ornetzer, M. S. (Dept. Biochemistry, Univ. Florida, ainesville, Fla. 32610); Stein, G. S. *Proc. Natl. cad. Sci. USA* 72(8):3119-3123; 1975.

hromatin was prepared from isolated nuclei of pro-iferating and differentiated cultures of C1300 ouse neuroblastoma cells. Differentiation was in-uced by serum withdrawal or treatment with dibuty-yl cyclic AMP. The ability to support DNA-depend-nt RNA synthesis in a cell-free system is three imes greater for chromatin from proliferating cells. istones isolated from proliferating and differen-iated cells were fractionated electrophoretically. he relative amounts of proteins present in the five ajor histone fractions were similar. In contrast, here were significant differences in the nonhistone hromosomal proteins synthesized and associated with he genome of proliferating and differentiating euroblastoma cells. Such differences are reflected y modifications in the electrophoretic banding pat-erns and in incorporation of [^{3}H]tryptophan into arious molecular wt classes of nonhistone chromo-omal polypeptides. This study demonstrates that hanges in the composition and transcriptional pro-erties of the genome of C1300 cells are associated ith the transition from proliferating to differen-iated state.

2334 CHROMATIN CHANGES DURING THE CELL CYCLE OF HeLa CELLS. (Eng.) Nicolini, C. (Temple Univ. Health Sci. Cent., Philadelphia, Pa); Ajiro, .; Borun, T. W.; Baserga, R. *J. Biol. Chem.* 250(9): 381-3385; 1975.

HeLa S_3 cells were synchronized by selective mitotic etachment, and chromatin was isolated from synchro-ized populations at various times during the cell ycle. The isolated chromatins were studied by cir-ular dichroism and for their ability to bind ethid-um bromide. Chromatin from cells in the middle of the S phase showed an increase in maximum ellipticity in the 250- to 300-nm region of circular dichroism spectra and an increased ability to bind ethidium bromide, when compared to chromatin isolated from mitotic cells. Chromatin from G_1 cells had values intermediate between mitotic and mid-S chromatins. The data show that the chromatin of HeLa cells under-go structural changes during the cell cycle that can be detected by circular dichroism and by ethidium bromide binding. The significance of these changes is still uncertain, although the evidence favors a correlation with transcriptional activity.

2335 ISOLEUCINE METABOLISM BY LEUKEMIC AND NORMAL HUMAN LEUKOCYTES IN RELATION TO CELL MATURITY AND TYPE. *(Eng.)* Burns, C. P. (Univ. Iowa Coll. Med., Iowa City). *Blood* 45(5):643-651; 1975.

The WBC of 19 patients with blood dyscrasias and of six normal subjects were isolated to compare the rate of protein synthesis by mature and immature WBC. Rates of protein synthesis of leukemic cells were correlated with the percentage of blast cells and isoleucine metabolism was studied. WBC were separated and incubated in 2 μCi [U-^{14}C] isoleucine for four hr. Protein precipitate was measured by ^{14}C-incorporation and counter scintillation. Thy-midine and uridine incorporation was determined by liquid scintillation spectrometry. Isoleucine was incorporated into protein at the rate of 2.0 to 33.4 nM/hr/10^8 WBC. Thymidine was utilized at rates from 11.0 to 310.2 M/hr/10^8 WBC. Lowest rates of isoleucine incorporation occurred in mature WBC of normal patients. Protein synthesis was highest in cells of patients with acute leukemia. Prepar-ations of immature cells had high rates of protein synthesis. Cell preparations with more blast cells had high rates of protein synthesis. Protein syn-thesis and thymidine incorporation were correlated since a high rate of protein synthesis was related to higher thymidine incorporation. Oxidative de-carboxylation of isoleucine and incorporation of ^{14}C from isoleucine into cell lipid was constant among all cell types studied. The author suggests that protein is synthesized in response to mitotic requirements. The data indicate that isoleucine metabolism occurs in a variety of WBC and that immature WBC incorporate protein more rapidly than mature WBC.

2336 STRUCTURAL DIFFERENCES IN FERRITINS FROM NORMAL AND MALIGNANT RAT TISSUES. *(Eng.)* Linder, M. C. (Dep. Nutr. Food Sci., Mas-sachusetts Inst. Technol., Cambridge); Moor, J. R.; Munro, H. N.; Morris, H. P. *Biochim. Biophys. Acta* 386(2):409-421; 1975.

The similarities and differences in subunit pri-mary structure of ferritin from normal and tumor tissue were determined. Normal tissue was obtained from adult female and newborn CDF rats. Buffalo and ACI rats were also used. Tumors studied in-cluded Morris hepatoma 3683F and 7800, and Morris kidney tumor MK-3. Tissues were obtained from ani-mals injected with 20 mg Fe as iron dextran. Fer-ritin was isolated and iron was removed by dialysis. The ratio of subunits was determined by electro-phoresis and spectrophotometry. Amino acid compo-sition, mapping of tryptic peptides, sulfhydryl groups and subunit determinations were made on fer-ritin of each tissue type. The amino acid composi-tion and tryptic peptide content of ferritin differed in each tissue type, except adult liver and iron-injected neonatal liver which were identical. Hepa-toma 3683F had the highest ratio of subunits and adult liver the lowest. There was no relation be-tween sulfhydryl groups and iron content of mole-cules. The same electrophoretic migration of iso-

ferretins did not indicate identical primary structure. Tumor ferritins are neither identical to neonatal liver or mature kidney ferritin, nor do they recapitulate them.

2337 AUTORADIOGRAPHIC LOCALIZATION OF GLYCOPROTEIN IN HUMAN BREAST CANCER CELLS MAINTAINED IN ORGAN CULTURE AFTER INCUBATION WITH [^{3}H]-FUCOSE OR [^{3}H]GLUCOSAMINE. (Eng.) Dermer, G. B. (Good Samaritan Med. Cent., Los Angeles, Calif.); Sherwin, R. P. *Cancer Res.* 35(1):63-67; 1975.

Explants of nine infiltrating duct carcinomas of the human female breast obtained minutes after mastectomy and maintained in organ culture were exposed to glycoprotein precursers L-[^{3}H]fucose and [^{3}H]-glucosamine to determine the cellular distribution of newly synthesized glycoprotein. Autoradiography with light and electron microscopes revealed glycoprotein sites. Some labeled explants were removed for autoradiographic analysis after incubation with a single isotope for 24 hr. The remainder were transfered to nonradioactive medium for 24 hr. After exposure to label, autoradiography with each isotope showed strong reactions over most tumor cells. Distribution of the label changed with time. Later samples showed a reduced reaction for Golgi apparatus, while the reactions of other organelles and ductular structures remained the same. In this *in vitro* system, human malignant breast epithelial cells were metabolically active and completed the oligosaccharide side chain of glycoprotein in the Golgi complex. Some of the newly synthesized glycoprotein may migrate to plasma membranes and lysosome-like bodies. Thus, many duct carcinomas of the breast exhibit secretory ability because some glycoprotein was shown to be secreted into intracellular and extracellular ductular structures.

2338 AMOUNTS OF ISOACCEPTING LYSINE tRNAs CHANGE WITH THE PROLIFERATIVE STATE OF CELLS. *(Eng.)* Juarez, H. (Dept. Biochemistry, Kansas State Univ., Manhattan, Kans. 66506); Juarez, D.; Hedgcoth, C.; Ortwerth, B. J. *Nature* 254(5498): 359-360; 1975.

The distribution of lysine tRNA isoacceptors was determined by RPC-5 chromatography in mouse cells in different states of proliferation: adult liver, whole embryo, and growing and quiescent primary cultures of embryonic cells. The results show that normal growing cells, like neoplastic cells (e.g., mouse leukemia and Morris hepatoma cells), contain an appreciable amount of $tRNA_4^{Lys}$. Species 4 was 24% of the total $tRNA^{Lys}$ in growing primary embryo cell cultures and 15% in growing embryonic cells not in tissue culture. In contrast to results with growing cells, the amount of $tRNA_4^{Lys}$ was 9% in quiescent primary embryo cells and only 3% in adult liver. Lysine tRNA isoacceptors 1, 2, and 5 were also detected in quiescent and growing mouse cells. $tRNA_2^{Lys}$ varied from 58% in adult liver to 35% in growing primary cultures of embryonic cells. It is concluded that the distribution of isoaccepting species of $tRNA^{Lys}$ in both normal and neoplastic cells varies with the proliferative state of cells.

2339 POLYPHASIC CHANGES IN INCORPORATION OF PRECURSORS INTO RIBONUCLEIC ACID OF OESTRADIOL-STIMULATED MAMMARY GLAND. (Eng.) Auricchio, F. (Cattedre di Istituzioni Di Patologia Generale e di Patologia Generale II, la Facolta di Medicine e Chirurgia, Universita di Napoli, S. Andrea Delle Dame 2, 80138 Napoli, Italy); Satriano, R.; Rotondi, A.; Quirino, A.; Bresciani, F. *Biochem. J.* 152(2):211-216; 1975.

The incorporation of [^{3}H]uridine into total RNA of ovariectomized mice that were treated with estradiol-17β was studied. Three weeks after ovariectomy, mammary glands (5th pair) of adult Swiss mice showed no significant decrease in weight, 20% of the original rate of incorporation of [^{3}H]-uridine into RNA (after a 30 min pulse), and 90% of the original rate of incorporation of L-[^{3}H]leucine into protein (after a 15 min pulse). A single injection of estradiol-17β (10 μg, ip) into these ovariectomized mice produced, during the next 17 hr, a series of discrete bursts of increased incorporation of [^{3}H]-uridine into mammary-gland RNA; the bursts, which were variable in height, reach peaks at about 1, 9, 12 and 16 hr after hormone administration; an increase was already detected at 15 min, the earliest time-point investigated; each burst lasted for about two hours. There was no significant stimulation of [^{3}H]uridine incorporation into RNA of liver and quadriceps femoris muscle. Nuclear incorporation of [^{3}H]uridine triphosphate into RNA of mammary gland *in vitro* was linear with time for up to 20 min at 15 C; it required cytosine triphosphate, guanidine triphosphate, and ATP and was inhibited by actinomycin D. Also, the incorporation was strongly inhibited by α-amanitin in high salt concentrations but only weakly in low salt concentrations, a result indicating that RNA polymerase II activity predominates in high salt, whereas RNA polymerase I activity predominates in low salt concentrations. Injection of estradiol-17β *in vivo* showed a definite increase in both RNA polymerase activities 30 min after estradiol-17β injection, the earliest time-point investigated, a higher increase at one hour, a decline at four hours, and again a large increase at 12 hr. These results generally agree with the changes in precursor incorporation into RNA measured directly in the animal, and suggest that changes in [^{3}H]uridine uptake into RNA are not precursor-pool-dependent.

2340 MODIFIED NUCLEOSIDES AND BIZARRE 5'-TERMINI IN MOUSE MYELOMA mRNA. (Eng.) Adams, J. M. (Walter and Eliza Hall Inst. Medical Research, Victoria 3050, Australia); Cory, S. *Nature* 255(5503):28-33; 1975.

Ten sequences representing the 5' termini of a large fraction of mouse myeloma messenger RNAs (mRNAs) are reported. In every sequence, the 5' terminal has 7-methylguanosine in a 5',5'-triphosphate linkage with ribosemethylated nucleotides; no derivatives of m^7G, such as the trimethylguanosine of the small nuclear RNAs have been detected. In the relative molar frequency of the oligonucleotides, certain sequences are much more abundant than oth-

ers; the most common sequence $m^7G^5ppp^{5'}CmpUp$ represents about 20% of the modified termine, and this and four other sequences constitute about 60% of these end groups. Nucleoside diphosphates (pNp) have also been detected in the mRNA digests, but the yields have been very variable and were lowest in the two mRNA preparations in which the highest yields of oligonucleotides were found. Poly(A)-containing mRNA from mouse myeloma cells contain at least eight kinds of modified nucleosides including 7-methylguanosine, N^6-methyladenosine, the four standard ribose-methylated nucleoside, and smaller amounts of two unidentified components. The various modified 5' termini in mRNA may assume different physical structures. This new form may prove to be a general feature in many eukaryotic cells and for a variety of viral mRNAs. Since the location of mRNA near the 3-termini of nuclear precursors is not firmly established the possibility exists that the 5'-terminal sequences in the mRNAs correspond to the 5' termini of primary transcripts.

2341 HETEROGENEOUS NUCLEAR RNA FROM LYMPHOCYTES OF CHRONIC LYMPHOCYTIC LEUKAEMIA: ADENYLATE-RICH AND DOUBLE-STRANDED REGIONS. (Eng.) Mansson, P. E. (Biokemi 1, Kemicentrum, Box 740, 220 07 Lund 7, Sweden); Holmquist, L.; Deutsch, A.; Norden, A. *Scand. J. Haematol.* 14:42-56; 1975.

Rapidly labeled, high-molecular wt nuclear RNA from lymphocytes of 11 patients with chronic lymphocytic leukemia (CLL) was analyzed for ribonuclease (RNase)-stable, adenylate [poly(A)]-rich, and double-stranded regions. The poly(A) content corresponds to 0.4-0.5% and the content of double-stranded sequences to 2-4% of the total nucleotides. Partial association of poly(A) segments with double-stranded regions was found by comparative analysis of ^3H-adenosine and ^3H-uridine labeled RNase-stable RNA before and after thermal denaturation. Partial degradation of rapidly labeled, high-molecular wt RNA occurred in CLL cases with a low WBC count. Compared with CLL lymphocytes, normal lymphocytes had a higher proportion of poly(A)-containing RNA binding to poly(U)-Sepharose but the same RNase-stable poly(A) content. This indicated a higher average size of poly(A)-bearing heterogeneous nuclear RNA (HnRNA). Gel electrophoresis of the RNase-stable poly(A) fraction bound to poly(U)-Sepharose revealed a higher proportion of larger molecules (100-200 nucleotides) in normal lymphocytes and a higher proportion of smaller molecules (20-40 nucleotides) in CLL lymphocytes. Thus, this finding of higher proportions of poly(A)-containing RNA in normal lymphocytes could be due to both a higher average size and to a larger proportion of poly(A)-bearing HnRNA. In CLL lymphocytes, the higher proportion of small poly(A)-containing molecules may indicate the presence of specialized regions in HnRNA absent in normals.

2342 ON THE MECHANISM FOR FORMATION OF RNA-DNA COMPLEXES FROM LYMPHOCYTES. (Eng.) Mendelsohn, J. (Univ. California, San Diego Sch. Medicine, La Jolla, Calif. 92037); Castagnola, J. M.; Goulian, M. *Biochim. Biophys. Acta* 407(3):283-291; 1975.

To test the possibility that RNA x DNA complexes form as artifacts of isolation procedures, association of RNA with nascent DNA from human diploid lymphocytes (line 8866) was studied. Nucleic acid extracts from cells pulse-labeled with [^3H]uridine contain RNA that banded near DNA in Cs_2SO_4 equilibrium density gradients. The amount of RNA bound to DNA was greatly reduced by repeated denaturation and equilibrium centrifugation. Substitution of calf thymus or *Escherichia Coli* DNA for lymphocyte DNA markedly reduced RNA banding with DNA. An apparently similar complex between RNA and DNA was formed in reconstruction experiments in which purified [^3H]uridine-labeled RNA was mixed with purified DNA. The association between RNA and DNA could be eliminated in the reconstruction experiments and greatly reduced in extracts from pulse-labeled cells by denaturation and equilibrium centrifugation in the presence of formaldehyde. These studies demonstrate that noncovalent bonding between RNA and DNA can account for most of the RNA with density close to that of DNA in Cs_2SO_4 gradients of nascent DNA preparations. The density of the residual DNA-associated RNA is consistent with a ribonucleotide content of 1/10 to 1/5 of the DNA molecule.

2343 CLUSTERING OF THE DNA SEQUENCES COMPLEMENTARY TO REPETITIVE NUCLEAR RNA OF HeLa CELLS. (Eng.) Melli, M. (Institut für Molekularbiologie II der Universität Zürich Winterthurerstr. 266A 8047 Zürich, Switzerland); Ginelli, E.; Corneo, G.; di Lernia, R. *J. Mol. Biol.* 93(1):23-38; 1975.

The hybridization profile of heterogeneous nuclear RNA from HeLa cells was studied across DNA density gradients. When heat-denatured heterogeneous nuclear RNA (HnRNA) was hybridized to fractions of higher molecular wt DNA from the HeLa cells, the pattern of hybridization did not follow the optical density with two main regions: one on the light and one on the heavy side of the main band. The DNA responsible for the hybridization was concentrated in limited regions of the density gradient, indicating that the sequences were clustered. The conditions used for hybridization allowed the detection of only those components in the RNA complementary to reiterated sequences in the DNA. The main part of the hybridization that gave a discrete region was the double-stranded DNA. The double-stranded RNA showed a pattern of hybridization across a DNA density gradient which was similar to that of total HnRNA. HnRNA contained a higher proportion of repeating sequences than cytoplasmic RNA and are concentrated double strand regions. Hybrids to fractions of DNA showed that more than one class of repeating sequences were represented in HnRNA. It is suggested that a considerable fraction of the sequences in the DNA which do form hybrids are clustered together in the genome and that the repeated sequences in HnRNA are complementary to clusters of repeated sequences in the DNA.

2344 ON THE FATE OF DNA SYNTHESIZING LYMPHOID BLOOD CELLS IN HODGKIN'S DISEASE. *(Eng.)* Schick, P. (Municipal Hosp. Munchen-Schwabing, West Germany); Trepel, F.; Begemann, H. *Scand. J. Haematol.* 14(1):17-23; 1975.

The fate of DNA synthesizing lymphoid cells of two patients with Hodgkin's disease was studied using autotransfusions of ^{3}H-thymidine-labeled blood. Patient T. E. received 2 l labeled blood within six hr and patient S. W. received 2.5 l within 8 hr. Blood samples were collected 5 min after each autotransfusion and 1, 5, 10, 20, 25, 30, 40, 50 and 60 hr after the last autotransfusion. WBC counts were made and, at 15 hr, small lymph nodes were removed for autoradiographic evaluation. Only 0.015% labeled cells were detected in the circulating blood of an expected 0.06%. This result may be due to an initial trapping of transfused cells. After two hr, autotransfused cells migrated to tissues. Mean grain count 1-25 hr after last autotransfusion was half that of autotransfused cells. Labeled blood lymphoid cells were formed by mitosis of transfused DNA synthesizing cells. By the 40th hr, labeled cells represented the second generation of transfused cells. A 25 hr generation time of proliferating lymphoid cells is typical of Hodgkin's patients. It is concluded that the DNA synthesizing lymphoid cells in the blood of Hodgkin's patients function as precursors of short-lived lymphocytes; they settle in the lymph nodes and spleen.

2345 CYTOPLASMIC DNA OF HEPATOMA TUMOR CELLS STUDIED BY ^{3}H-ACTINOMYCIN D BINDING. (Eng.) Aczel, J. (Dept. Biological Sciences, Sir George Williams Univ., Montreal, Quebec H3G 1M8, Canada); Enesco, H. E. *Experientia* 31(3):360-361; 1975.

The cytoplasmic DNA content of mouse hepatoma tumor cells was compared with that of normal mouse liver cells. Incubation with ^{3}H-actinomycin D (^{3}H-AD) followed by radioautography were the methods used. The tumor was a hepatoma maintained by serial transplantation in C57L/J mice. No significant differences were observed between RNase-treated sections and nontreated sections indicating that the presence or absence of RNA has no effect on ^{3}H-AD binding. ^{3}H-AD binding was more than two times greater in the cytoplasm of hepatoma cells than in the cytoplasms of normal C57L/J liver cells. Mitochondrial DNA (mDNA) is the best documented class of cytoplasmic DNA. Differential ^{3}H-AD binding to tumor mDNA or an increase in the amount of mDNA in the hepatomas could account for the results. This study provides additional proof that cytoplasmic DNA is elevated in hepatoma cell as compared to their normal counterparts.

2346 DNA CONTENTS OF INDIVIDUAL CELLS FROM SQUAMOUS CELL CARCINOMAS OF THE LARYNX AND TONGUE. (Eng.) Jakobsson, P. A. (Radiumhemmet, Karolinska Sjukhuset, S-104 01, Stockholm 60, Sweden); Killander, P. D.; Silfversward, C.; Wersall, J. *Laryngoscope* 85(10):1701-1706; 1975.

Scrapings were obtained from three upper respiratory squamous cell carcinomas, and histologic grading of malignancy was evaluated by quantitating cellular amounts of DNA. Scrapings were obtained from one normal individual, two patients with glottic carcinoma of the larynx and one with carcinoma of the tongue. Cells were washed and centrifuged for five minutes at 1,400 rpm to facilitate attachment to the hemocytometer slides, then stained by the Feulgen method with parallel preparations by standard cytologic techniques. The Feulgen DNA of individual cells was determined in a rapid scanning microspectrophotometer, and a clinical judgement was made prior to scanning. Cells from normal buccal mucosa were exclusively superficial epithelium with a modal distribution having a mean Feulgen DNA value of 14.7 U, corresponding to a presynthetic amount of DNA. Cells from the lingual carcinoma were similar, with a mean DNA value of 11.6 U. Cells from the two laryngeal carcinomas exhibited much intercellular variations, with mean values of 26.5 and 15.7 U, but with trimodal distribution of the cells. Peak DNA values for the two cases occurred at 12.4 and 11.6 U, 27.2 and 21.2 U, and 32.6 and 24.0 U, corresponding to three populations of cells: normal, suspicious, and malignant, respectively. Some malignant and suspicious cells were also found within the "normal" population by DNA determination. This study demonstrates heterogeneous patterns of DNA content of individual cells in squamous cell carcinomas of the larynx and low values in the case of tongue carcinoma. These values can be related to histological malignancy point scoring when the type of cells measured is considered. This is demonstrated best in the case of tongue carcinoma in which only normal cells were found by the technique, while analysis of the infiltrative portion of the tumor may have been more productive. The authors indicate that cells with increased DNA amounts had malignant or suspicious morphology and that these increased amounts indicate cell proliferation and/or the existence of polyploid cells.

2347 DNA SYNTHESIS IN MEMBRANE-DENUDED NUCLEI AND NUCLEAR FRACTIONS FROM HOST LIVER AND MORRIS HEPATOMAS. (Eng.) Coetzee, M. L. (Univ. Pittsburgh Sch. Medicine, Pittsburgh, Pa. 15261); Spangler, M.; Morris, H. P.; Ove, P. *Cancer Res.* 35(10):2752-2761; 1975.

Incorporation of [^{3}H]TTP into membrane-denuded nuclei and fractions of these nuclei from host liver and Morris hepatomas was compared. Treatment of sucrose nuclei with Triton X-100 removed 95% of the phospholipids and 15-20% of the protein. These membranes-denuded nuclei remained physically stable. The Triton X-100-extracted nuclei incorporated label into their DNA in a nuclear-incorporating system similar to sucrose nuclei with their membranes intact. Triton X-100-treated nuclei from hepatoma 7777 incorporated six times more label, and those from hepatoma 7800 incorporated three times more label than did Triton X-100-treated host liver nuclei. Nuclei from the three sources incorporated more label when exogenous DNA was added to the incubation system, but the difference in incorporation between the hepatoma nuclei and the host liver nu-

ei disappeared. When Triton X-100-treated nuclei, epared from a tumor-bearing male Buffalo rat given jections of [^{3}H]thymidine for ten minutes were actionated on sucrose gradients after disruption high Mg^{2+} concentration (2 μM $MgCl_2$), the fracons from hepatoma 7777 nuclei contained six times much label as the host liver nuclear fractions. clear fractions prepared from unlabeled hepatomas host livers had DNA polymerase activity. The acvity, however, was the same in fractions prepared om hepatoma 7777 or host liver nuclei. It is sugsted that the nuclear membrane does not play an portant role in nuclear DNA synthesis. The ineased incorporation found with hepatoma nuclei y be dependent on a physical or chemical arrangent of components within the nucleus, and not solely different enzyme levels.

48 ABSENCE OF SPECIFICITY IN INHIBITION OF DNA REPAIR REPLICATION BY DNA-BINDING ENTS, COCARCINOGENS, AND STEROIDS IN HUMAN CELLS. Eng.) Cleaver, J. E. (Lab. of Radiobiology, Univ. California, San Francisco, Calif. 94143); Painter, B. *Cancer Res.* 35(7):1773-1778; 1975.

e effects of various agents on DNA synthesis and DNA pair replication were investigated. DNA-binding ents tested were chloroquine and quinacrine; carcinens were vitamin A alcohol, Tween 80, anthraline, d phorbol myristate acetate; steroids were diethylilbestrol, norethindrone, progesterone, and estraiol. The nonspecific chemicals potassium cyanide, enethyl alcohol, and dimethyl sulfoxide were also sted. Human lymphocyte suspensions prepared from eshly drawn heparinized blood and HeLa S3 cells re employed. Semiconservative DNA replication was termined by measuring [^{3}H]thymidine incorporation, d DNA repair was measured by the Gaudin method. In irradiated HeLa cultures and lymphocytes, incubation [^{3}H]bromodeoxyuridine produced exclusively hybrid nsity double-stranded DNA, indicating semiconservaive DNA replication. Irradiation of HeLa cultures ppressed semiconservative replication and induced pair replication, yet both replications occurred in e same cultures. However, only repair replication curred in irradiated WBC cultures. Using unstimuated WBC suspensions to measure the effect of chemials added concurrent with [^{3}H]thymidine, it was ound that repair was never inhibited to a greater xtent than was semiconservative replication. HeLa ultures were less sensitive than WBC to some chemials, and unaffected by steroids. The lack of speciic effects on DNA repair synthesis negates the hypohesis of carcinogenesis *via* DNA repair inhibition. t suggested that correlating inhibition of DNA repair ith potential chemotherapeutic values of agents is isleading and futile.

349 FLUORESCENCE POLARIZATION AND VISCOSITIES OF MEMBRANE LIPIDS OF 3T3 CELLS. (Eng.) uchs, P. (Center for Cancer Res., Massachusetts nst. Technology, Cambridge, Mass. 02139); Parola, .; Robbins, P. W.; Blout, E. R. *Proc. Natl. Acad. Sci. USA* 72(9):3351-3354; 1975.

luorescence polarization and microviscosity values of the lipid bilayer of membranes of normal, transformed and revertant 3T3 cells were obtained using a fluorescent hydrocarbon, 1,6-diphenyl-1,3,5-hexatriene. Following 60 min of labeling, fluorescence polarization values of normal cells were significantly lower than those of cells transformed with either the polyoma virus or the simian virus 40. Fluorescence polarization values of Py6R$_1$ cells, a revertant line isolated from 3T3Py6 cells by a fluorodeoxyuridine procedure, were also lower than those obtained with normal cells. Microviscosity values, calculated from mean fluorescence anisotrophy values were significantly higher in transformed cells and revealed a 50% increase. Possible correlations between the bilayer viscosity and the mobility of the receptor in the membrane are discussed.

2350 LIPIDS OF CULTURED HEPATOMA CELLS: VI. GLYCEROLIPID AND MONOENOIC FATTY ACID BIOSYNTHESIS IN MINIMAL DEVIATION HEPATOMA 7288 C. (Eng.) Wiegand, R. D. (Univ. Missouri Sch. Med., Columbia); Wood, R. *Lipids* 10(3):194-201; 1975.

1-^{14}C-acetic acid, 1-^{14}C-palmitic acid, or 1-^{14}C-stearic acid was incubated with minimal deviation hepatoma 7288C cells grown in culture to assess: *de novo* fatty acid synthesis, oxidation, desaturation, and elongation of saturated fatty acids, as well as the ability of media fatty acids to serve as precursors of cellular glycerolipids. Distribution of radioactivity in the individual lipid classes and the various fatty acids of triglyceride, phosphatidyl choline, and phosphatidyl ethanolamine was determined. The radioactivity was analyzed by reductive ozonolysis. Only small amounts of the labeled substrates were oxidized to carbon dioxide. Except for labeled stearic acid, which also was incorporated heavily into phosphatidyl inositol and phosphatidyl serine, most radioactivity was recovered in triglyceride, phosphatidyl choline, and phosphatidyl ethanolamine. Synthesis of cholesterol and long chain fatty acids from labeled acetic acid demonstrated that these cells can perform *de novo* synthesis of fatty acids and cholesterol. Both labeled palmitic and stearic acids were desaturated to the corresponding Δ9 monoenes, and palmitic and palmitoleic acids were elongated. The hexadecenoic acid fraction isolated from triglyceride, phosphatidyl choline, and phosphatidyl ethanolamine, when acetic or palmitic acid was the labeled substrate, showed that greater than 70% of the labeled acids were the Δ9 isomer. Radioactivity of the octadecenoic acid fraction derived from labeled acetic or palmitic acids was nearly equally divided between the Δ9 isomer, oleic acid, and the Δ11 isomer, vaccenic acid. Desaturation of labeled stearic acid produced only oleic acid. These data demonstrate that the biosynthesis of vaccenic acid in these cultured neoplastic cells proceeds via the elongation of palmitoleic acid. The relatively high level of vaccenic acid synthesis in these cells suggests that the reported elevation of "oleic acid" in many neoplasms may result from increased concentration of vaccenic acid.

2351 THE TYROSINASE ACTIVITY OF MELANOSOMES FROM THE HARDING-PASSEY MELANOMA: THE ABSENCE OF A PEROXIDASE COMPONENT *IN VITRO*. (Eng.) Mufson, R. A. (Div. Biol. Med. Sci., Brown Univ., Providence, R.I.). *Arch. Biochem. Biophys.* 167(2): 738-743; 1975.

Melanosomes were isolated from the Harding-Passey melanoma with a density gradient technique. Using the Pomerantz radioassay for tyrosinase activity, it was found that these isolated melanosomes could hydroxylate tyrosine in the presence of catalase sufficient to deny the enzyme any hydrogen peroxide. The rate of hydroxylation was unaffected by the presence of exogenous hydrogen peroxide. Tyrosinase activity could be suppressed by preincubation in diethyldithiocarbamate followed by removal of this inhibitor before enzyme assay. Attempts to regain enzymatic activity, however, by addition of copper II ions were unsuccessful. No peroxidase activity could be detected on the isolated granules, and evidence for a peroxidase inhibitor on the granules was found when aliquots of melanosomes were mixed with horse radish peroxidase. The peroxidase activity present in a 20% homogenate of mouse muscle did not demonstrate any tyrosinase activity with the Pomerantz assay even in the presence of hydrogen peroxide. It is concluded from these studies that there is tyrosinase on these melanosomes which is capable *in vitro* of hydroxylating tyrosine without any contribution from an active peroxidase.

2352 INABILITY OF MURINE MELANOMA MELANOSOMAL "TYROSINASE" (L-DOPA OXIDASE) TO OXIDIZE TYROSINE TO MELANIN IN POLYACRYLAMIDE GEL SYSTEMS. (Eng.) Edelstein, L. M. (St. Vincent Hosp., Worcester, Mass.); Cariglia, N.; Okun, M. R.; Patel, R. P.; Smucker, D. *J. Invest. Dermatol.* 64(5):364-370; 1975.

The enzyme "tyrosinase" (L-dopa oxidase) was separated from trypsin-treated melanosomes of B-16 mouse melanoma in C57BL/6J mice by polyacrylamide gel disc electrophoresis. Gels were incubated with various tyrosine/L-dopa ratios from 1:1 to 40:1 and controls of L-dopa only. In the 7.7 mM dopa incubation, additional melanin bands were noted. There was no increase in melanin synthesis in the presence of tyrosine when compared with incubations in corresponding concentrations of dopa alone. Tyrosine inhibition of melanin synthesis occurred in tyrosine/dopa ratios greater than 2:1. The results thus demonstrate the inability of so-called "tyrosinase" to convert tyrosine to melanin; since the enzyme readily converts L-dopa to melanin, it seems more reasonable to term this enzyme an L-dopa oxidase.

2353 BIPHASIC INDUCTION OF ORNITHINE DECARBOXYLASE AND PUTRESCINE LEVELS IN GROWING HTC CELLS. (Eng.) McCann, P. P. (Merrell International Res. Center., 6700 Strasbourg, France); Tardif, C.; Mamont, P. S.; Schuber, F. *Biochem. Biophys. Res. Commun.* 64(1):336-341; 1975.

The relationship between putrescine, its biosynthetic enzyme ornithine decarboxylase (ODC), and cell division was studied in rat hepatoma cells (HTC). ODC activities were followed through two generations of HTC cells (48 hr) by measuring $^{14}C\text{-}CO_2$ released from ^{14}C-DL-ornithine in cell extracts. A biphasic induction of ODC with concomitant increases of intracellular putrescine occurred during each generation period. In nongrowing cells, only one broad peak of ODC activity was seen after dilution of cells into medium without serum. Intracellular putrescine levels did not increase, suggesting that regulation of putrescine biosynthesis might also depend on substrate availability. To estimate the degree of synchrony in growing cells after dilution into fresh medium, ^3H-thymidine incorporation into DNA was measured over a 24-hr period. Maximal incorporation into DNA was at 10-11 hr. Analysis of the HTC cell cycle indicated that the two bursts of ODC activity occurred in late G_1 and G_2 just before the S phase of DNA synthesis. The data demonstrate that while ODC activity can be induced in nongrowing cells, only growing and dividing HTC cells show biphasic induction and corresponding increased putrescine levels per generation time.

2354 INCREASE IN RAT LIVER RIBONUCLEASE INHIBITOR LEVELS DURING THE NEONATAL PERIOD. *(Eng.)* Liu, D. K. (Pennsylvania State Univ. Coll. Med., Hershey, Pa.); McKee, E. E.; Fritz, P. J. *Growth* 39(1):167-175; 1975.

Alkaline RNase activity in nuclear, mitochondrial and cytoplasmic fractions of rat liver, and cytoplasmic RNase inhibitor levels in rat liver were examined in male Sprague-Dawley rats (1-56 days old). Microsomal fractions were prepared from excised rat liver homogenate. Dialyzed ribosomal RNA was obtained from rats fasted for 15 hr. Free alkaline RNase and latent RNase activity was assayed and measured as the amount of enzyme that produced 1 μM of nucleotide residues. RNase inhibitory activity was defined as the amount of inhibitor necessary for 50% inhibition of 3 ng bovine pancreatic RNase. Free RNase activity of nuclear and mitochondrial fractions was high at birth and decreased by days 25 and 4, resp. RNase activity in the cytoplasmic fraction and the inhibitor-bound latent cytoplasmic RNase was high in newborn rats and declined with age. The RNase activity of the microsomal fraction and young adults was low; RNase inhibitor activity in cytoplasmic fraction was lowest in one-day-old animals. Reduction of RNase activity by an increase in RNase inhibitor activity allows a reduction in RNA degradation resulting in increased cellular RNA.

2355 ALTERATION OF GLYCINE *N*-METHYLTRANSFERASE ACTIVITY IN FETAL, ADULT, AND TUMOR TISSUES. (Eng.) Heady, J. J. (Univ. Colorado Med. Cent., Denver); Kerr, S. J. *Cancer Res.* 35(3):640-643; 1975.

In order to correlate the appearance of the glycine *N*-methyltransferase activity in developing embryonic tissue with the impact of that enzyme on transfer RNA methyltransferase activity, glycine methyltrans-

erase activity was examined in fetal and adult organs, as well as in several rodent hepatomas. In fetal rabbit liver, the activity first appeared at a low level at about 20 days postfertilization and rose to high levels after birth, reaching maximum in the adult liver. In fast-growing hepatomas, the activity could not be detected by either enzymatic or immunological assay. It could be detected in the slower-growing hepatomas, but in considerably diminished levels compared with that of normal adult rat liver. Immunoassays gave no evidence for inactive forms of the enzyme in the tissues that had no enzymatic activity. Transfer RNA methyltransferase assays carried out simultaneously showed an inverse relationship to the glycine *N*-methyltransferase activity. The levels of transfer RNA methyltransferase activity were high in fetal and tumor tissues and lower in normal adult tissues. These results demonstrate a significant difference in the physiology of fetal and tumor tissue *versus* normal tissue involving the system that governs the homeostasis between *S*-adenosylmethionine and *S*-adenosylhomocysteine, and particularly the availability of *S*-adenosylmethionine to the transfer RNA methyltransferases.

2356 PLASMA MEMBRANE-ASSOCIATED INCREASE IN GUANYLATE CYCLASE ACTIVITY IN REGENERATING RAT LIVER. (Eng.) Goridis, C. (CNRS-CBM4, 13274 Marseille-Cedex 2, France); Reutter, W. *Nature* 257(5528):698-700; 1975.

Cyclic guanosine monophosphate (cGMP) formation was measured in homogenated liver from female Buffalo rats after partial hepatectomy to investigate guanylate cyclase activity in regenerating liver. Two-thirds of the liver was removed from the experimental rats, and sham-operated rats served as controls. Liver homogenates were prepared from rats sacrificed at 2.5, 16, 24, 48, 72, 96, and 168 hr post-hepatectomy. Guanylate cyclase activity was assayed by standard techniques except that 0.1 mM papaverine was included to inhibit cyclic nucleotide phosphodiesterase activity, and the eluate of Al_2O_3 columns was further chromatographed on Dowex 1 x 8. Guanylate cyclase activity began to rise at 16 hr post-hepatectomy (16 pM cGMP/min/mg protein), peaked at 24 hr (24 pM/min/mg protein), and remained significantly elevated until 72 hr (20 pM/min/mg protein), after which it returned to normal values. While this enzyme was mainly detectable in the soluble fraction in quiescent liver (28.9 pM/min/mg protein), the major increase in activity occurred in the plasma membrane fraction after partial hepatectomy (141 pM/min/mg protein) as compared to controls (12.1 pM/min/mg protein). Guanylate cyclase activity was also investigated in four hepatoma lines (Morris hepatomas 7777, 9618 A_2, 7800, 9120); enzyme activity was increased in only one (9120, 25.6 pM/min/mg protein). This study indicates that guanylate cyclase activity is increased in regenerating liver and that this increase occurs in the plasma membrane fraction. The authors suggest that this normally functioning guanylate cyclase is essential for growth regulation.

2357 CYTOPLASMIC POLY(ADP-RIBOSE) POLYMERASE DURING THE HeLa CELL CYCLE. (Eng.) Roberts, J. H. (Dept. Biology, Catholic Univ. America, Washington D. C. 20064); Stark, P.; Giri, C. P.; Smulson, M. *Arch. Biochem. Biophys.* 171(1): 305-315; 1975.

The biosynthesis of poly(ADP-ribose)polymerase on cytoplasmic ribosomes during the time sequences of the cell cycle was investigated. Poly(ADP-ribose) polymerase, which has reportedly been confined to the nucleus of eukaryotic cells, was found in the cytoplasm of HeLa cells. The enzyme activity was stimulated more than 30-fold by the addition of both DNA and histones. These two macromolecules were absolutely necessary for maximal activity, and they acted in a synergistic manner. The product of the reaction was characterized as poly(ADP-ribose) by its acid insolubility, its insensitivity to hydrolysis by DNase, RNase, spleen phosphodiesterase or Pronase and by release of 5'-AMP and 2'(5"-phosphoribosyl)-5'-AMP by incubation with snake venom phosphodiesterase. A covalent attachment between histone F1 and poly(ADP-ribose) was established by using the cytoplasmic enzyme. The enzyme was found to be primarily associated with ribosomes, both free ribosomes and those found in polysomes. Inhibition of protein synthesis in the intact cell reduced the level of activity in the cytoplasm. The enzyme could be removed from the ribosomes by centrifugation through sucrose gradients containing 0.6 M ammonium chloride. A relationship between this enzyme and DNA replication is suggested by the fact that the specific activity in the cytoplasm parallels the rate of DNA synthesis during the HeLa cell cycle.

2358 PROTEASES DURING THE GROWTH OF EHRLICH ASCITES TUMOR. I. THE FIBRINOLYSIN SYSTEM. (Eng.) LeBlanc, P. P. (Laval Universite, Ecole de Pharmacie, Quebec, 10e, Canada); Back, N. *J. Natl. Cancer Inst.* 54(4):881-886; 1975.

Component levels of the fibrinolysin system in the plasma and ascitic fluid of Swiss mice bearing Ehrlich ascites tumor cells were determined during a 15-day tumor growth time phase. During tumor growth, the concentration of plasminogen in the ascitic fluid decreased inversely to the total packed cell volume. Free plasmin was not present in the ascitic fluid, nor was there any measurable plasminogen activator activity. Both antiplasmin activity and fibrinogen levels present in the fluid decreased during tumor growth. The nucelar and mitochondrial-microsomal subcellular fractions of the tumor cell produced zones of lysis, confirming the presence of plasminogen activator. No significant changes in the above parameters occurred in the plasma during the tumor growth period studied. The plasminogen activator activity exhibited by the nuclear and mitochondrial-lysosomal fractions of the tumor cell would support the hypothesis that plasminogen is converted to plasmin which, after complexing with the antiplasmim, consumes fibrinogen. Alternative interpretations of the changes in component levels include possible production and destruction of factors by the growing

cell population or transudation of the factors with destruction and consumption by accompanying factors in the fluid.

2359 MEDIATION OF SERUM-INDUCED CHANGES IN CYCLIC NUCLEOTIDE LEVELS IN CULTURED FIBROBLASTS BY CYCLIC NUCLEOTIDE PHOSPHODIESTERASE. (Eng.) Pledger, W. J. (Univ. Texas Medical Sch., Houston, Tex. 77025); Thompson, W. J.; Strada, S. J. *Nature* 256(5520):729-731; 1975.

The hypothesis that substrate affinities of cyclic AMP phosphodiesterase activities are altered in response to conditions affecting cell growth was tested by measuring enzyme activity after addition of 10% fetal calf serum to quiescent BHK 21/c13 fibroblasts (cultured in media containing 1% fetal calf serum). Cyclic AMP phosphodiesterase activity, measured at a 1 μM cyclic AMP concentration, increased 4-5-fold within the first 15 min of serum addition. The total cyclic guanosine monophosphate (GMP) phosphodiesterase activity, measured at a cyclic GMP concentration of 1.25 μM, was reduced 3-fold at this time. The cyclic AMP phosphodiesterase of cells incubated in 1% serum had an apparent K_m of 15 M cyclic AMP. The enzyme from cells incubated for an additional 30 min in 10% serum showed activity with anomalous kinetic behavior and apparent K_m values of 3 and 20 M cyclic AMP. The phosphodiesterase activities of cells maintained in 1% serum in the presence of cycloheximide (0.2 mM) had a lower affinity (higher apparent K_m) for cyclic AMP than did cells receiving 10% serum in the presence of cycloheximide. No differences in kinetic profiles were noted between control quiescent cells and those that received cycloheximide. These results show that the rapid change in substrate affinity of cyclic AMP phosphodiesterase induced in quiescent cells by growth-promoting serum concentrations takes place in the absence of protein synthesis. A decrease in cyclic AMP levels within 15 min after the addition of 10% serum to quiescent cells was correlated with the increase in cyclic AMP phosphodiesterase activity measured at 1 μM cyclic AMP. These data confirm that growth-promoting concentrations of serum result in a concomitant, rapid and transient change in intracellular cyclic nucleotide levels. The substrate affinity, as well as the amount of phosphodiesterase, could thus be involved in the regulation of cyclic nucleotide concentrations.

2360 CYCLIC AMP PHOSPHODIESTERASE ACTIVITY IN THREE MORRIS HEPATOMAS. (Eng.) Criss, W. E. (Univ. Florida Coll. Med., Gainesville); Morris, H. P. *Enzyme* 20(2):65-70; 1975.

The presence of multiple molecular forms of cyclic AMP phosphodiesterase activity in a series of Morris hepatomas was demonstrated. Cyclic AMP phosphodiesterase from the livers of hepatoma-inoculated Buffalo strain rats was fractionated into four peaks of activity (1 mM/*l* cyclic AMP) with isoelectrofocusing column chromatography. The major two liver peaks (high K_m enzymes) decreased with increasing growth rate while the minor two liver peaks (low K_m enzymes) increased in one fast growing Morris hepatoma. There was also less total phosphodiesterase activity in the fast growing hepatoma. The results may suggest that there is a shift in the cytoplasmic distribution of high K_m and low K_m cyclic AMP phosphodiesterases during hepatoma dedifferentiation.

2361 STUDIES ON CYCLIC AMP METABOLISM IN HUMAN EPIDERMOID CARCINOMA (HEp-2) CELLS. (Eng.) Kelly, L. A. (Sch. Med., Univ. Massachusetts, Worcester); Butcher, R. W. *Metabolism* 24(3):359-368; 1975.

Catecholamine-sensitive adenyl cyclase activity in human epidermoid carcinoma (HEp-2) was studied in intact cells and in cell homogenates. Homogenates showed a small but significant accumulation of cyclic AMP even without stimulatory agents. Ten μM epinephrine and 10 μM NaF, however, increased the accumulation of AMP six- and twelve-fold, resp. On the other hand, little cyclic AMP was accumulated in intact cells in either the presence of absence of epinephrine. Furthermore, addition of theophylline or exogenous ATP-Mg^{2+}(3 mM) caused no significant increase in cyclic AMP in epinephrine-treated intact cells. No change was observed at any population density tested or at any incubation time between 5 and 120 min. A transfer of medium to responsive SV40-transformed fibroblasts yielded a normal fibroblast response, indicating that the epinephrine was not degrated by the intact HEp-2 cells. In the presence of 2 mM phosphodiesterase inhibitor, namely, 1-methyl, 3-isobutylxanthine (MIX), cyclic AMP increased significantly with 0.3 μM epinephrine and reached a maximum with 10 μM. In the presence of MIX, maximum amounts of cyclic AMP were released within 5 min of a 60-min incubation period. Norepinephrine caused less cyclic AMP activity than either epinephrine or isopropylnorepinephrine at 10 μM when combined with MIX. On the basis of these experiments, the effects of hormones on adenyl cyclase activity cannot safely be extrapolated to changes in intracellular cyclic AMP levels.

2362 LACK OF STEROIDOGENIC RESPONSE TO CYCLIC AMP AND ACTH IN ADRENAL CELLS FROM RATS BEARING THE $MtTF_4$ TUMOR. (Eng.) Rivkin, I. (Squibb Inst. for Medical Res., P.O. Box 4000, Princeton, N.J. 08540); Chasin, M. *Arch. Int. Pharmacodyn. Ther.* 217(2):309-321; 1975.

Adrenal cell suspensions prepared from rats bearing the transplantable mammotropic pituitary tumor $MtTF_4$ failed to increase corticosterone production when exposed to adenosine 3', 5'-cyclic monophosphate (cyclic AMP) or ACTH, placing the defect in these adrenals beyond the ACTH receptor site. Adrenal cells from normal rats responded well to these stimuli. Adrenal cyclic AMP phosphodiesterase prepared from the tumor bearing rats appeared to have normal specific activity and inhibition profile with theophylline. Exposure of the $MtTF_4$ adrenal cells to 1,2-^{3}H-cholesterol in the presence of either cyclic AMP or ACTH did not result in an increase in radioactively labeled corticosterone. An increase of two-folds in labeled corticosterone was found in adrenal cells from normal rats similarly treated.

2363 DIFFERENTIAL EFFECT OF HORMONES ON MACROMOLECULAR SYNTHESIS AND MITOSIS IN CHICK EMBRYO CELLS. (Eng.) Baseman, J. B. (Sch. Medicine, Univ. North Carolina, Chapel Hill, N.C. 27514); Hayes, N. S. *J. Cell Biol.* 67(2/Part 1):492-497; 1975.

The differential effect of hormones on macromolecular synthesis and mitosis in chick embryo cell cultures (prepared from 17-day-old embryos) was studied. Cells were grown to confluency and radiolabeled with uridine, thymidine or mixtures of amino acids. Hydrocortisone treatment (0.5 μg/ml) was either ineffective or depressed the synthesis of RNA, protein and DNA in monolayers of chick cells. However, hydrocortisone plus insulin (0.4 μg/ml) significantly increased [^{3}H]thymidine incorporation into trichloroacetic acid-precipitable material and also increased DNA synthesis, although mitosis was not apparent. Eight percent of the fibroblasts were actively engaged in DNA synthesis after insulin stimulation, while 14% of the cells synthesized DNA after exposure to insulin plus hydrocortisone. The addition of 3% calf serum to cells previously exposed to insulin plus hydrocortisone caused a significant increase in the mitotic index. Protein and RNA synthesis rates were decreased in cells incubated with insulin plus hydrocortisone when compared to monolayers treated only with insulin. The combined effects of insulin and hydrocortisone appeared to be both synergistic and antagonistic, depending on the specific macromolecular event. It is suggested that insulin and hydrocortisone together furnish the information for the initiation of mitosis, but require regulatory assistance from serum. The results indicate that the physiologic role of insulin may not be mitogenic. Nuclear autoradiography and the percent mitosis of the cells indicate that cell heterogeneity is reflected in the metabolic responses of subpopulations to specific regulatory signals.

2364 DESMOSTEROL IN RAT CENTRAL AND PERIPHERAL NERVOUS SYSTEMS DURING NORMAL AND NEOPLASTIC GROWTH. (Eng.) Weiss, J. F. (New York Univ. Medical Center, New York, N. Y. 10016); Cravioto, H.; Ransohoff, J. *J. Natl. Cancer Inst.* 54(3):781-783; 1975.

Tumors induced at specific sites in the rat central nervous system (CNS) and peripheral nervous system (PNS) were analyzed for sterol composition. The desmosterol content of developing cerebrum and trigeminal nerve was compared. The tumors were induced in various strains of rats by either transplacental treatment with ethylnitrosourea (ENU) of monthly i.v. administration of methylnitrosourea (MNU) to adult rats. The method used for sterol analysis involved saponification of the sample in 1 N alcoholic potassium hydroxide extraction of the nonsaponifiable material and separation of trimethylsilyl ether derivatives of the sterols by gas-liquid chromatography. Desmosterol diminished as the trigeminal nerve matured and increased levels were observed again only in definitely neoplastic tissue. A small but consistent increase in desmosterol was found in neurinomas of the trigeminal nerve. The average increase in desmosterol was greater in intracerebral tumors than in intracranial neurinomas. The highest amount of desmosterol was observed in L-cell mouse fibroblasts grown in tissue culture in a medium containing delipidized serum, though desmosterol was not detected in significant quantities in eight other normal and neoplastic cell lines. Since this study showed that the desmosterol level in myelinating trigeminal nerve is not very high, the myelination sequence in neural tissue does not seem inherently related to high desmosterol levels. Specific factors that might influence desmosterol accumulation in the CNS are unclear. Differences between the lipid composition and metabolic activity of PNS and CNS myelin may be related to the characteristics of oligodendroglial and Schwann cells from which CNS and PNS myelin are formed.

2365 ELEVATED SERUM LEVELS OF ESTROGEN AND PROLACTIN IN DAUGHTERS OF PATIENTS WITH BREAST CANCER. (Eng.) Henderson, B. E. (Edmondson Research Building, 1840 N. Soto St., Los Angeles, Calif. 90032); Gerkins, V.; Rosario, I.; Casagrande, J.; Pike, M. C. *N. Engl. J. Med.* 293(16):790-795; 1975.

Demographic risk factors in sisters and daughters of 150 patients with breast cancer were compared to those of controls matched for age (within one year) and marital status. Plasma hormone levels in 36 teen-age daughters of patients and 31 controls were also studied to ascertain whether an "abnormal" hormone pattern underlies these risk factors. The patients' sisters had, on the average, menarche four months earlier and first full-term pregnancy 12 mo later than the controls. The patients' daughters did not show these differences -- apparently owing to low fertility in the patients with early menarche. The patients' daughters had higher 22nd-day estradiol-plus-estrone levels than controls (24.4 *versus* 19.1 ng/100 ml, $P<0.05$). Sixth-day prolactin was also elevated (19.0 *versus* 14.2 ng/100 ml, $P<0.05$). About half the patients' daughters could clearly be distinguished from the controls' daughters by means of the sixth-day information on both estrogens and prolactin. Hypersecretion of these hormones may be important factors in breast cancer.

2366 PROSTAGLANDIN SYNTHESIS INHIBITION: EFFECT ON BONE CHANGES AND SARCOMA TUMOR INDUCTION IN BALB/c MICE. (Eng.) Strausser, H. R. (Dept. Zool. Physiol., Rutgers Univ., Newark, N.J.); Humes, J. L. *Int. J. Cancer* 15(5):724-730; 1975.

The effect of indomethacin on the growth and prostaglandin (PG) content of Moloney sarcoma virus (MSV) tumors in 6-wk-old immunocompetent Balb/cJ mice was studied. After mice were inoculated with the virus, they were injected sc with 0.1 ml indomethacin solution every second day. Two control groups were given either virus only, or indomethacin only. PG measurements, histological preparations, bone examinations, and serum calcium measurements were usually made at the peak of tumor growth (about 12 days). The level of the hormone increased with the tumor diameter and decreased with tumor regression. At the peak of tumor size the tibial bones were considerably de-

formed, suggesting osteoclastic activity. The systemic calcium level was not elevated, indicating possible release of calcium into the local tumorous area. In mice treated with indomethacin, the tumors failed to develop and PG levels were markedly lower. Tibial bones in treated mice were similar to those of control, nontumorous mice. PG levels of DBA/1J mice bearing extensive Cloudman S91 melanoma were not elevated and no bone deformation was seen. When contrasted with studies of immuno-depressed mice, the results suggested that indomethacin acts in conjunction with, and possibly restores the PG-induced depression of the immune system in preventing tumor development. Indomethacin, by suppressing the PG-mediated calcium release from bone, could be operative in inhibiting tumor growth. It is concluded that these tumors elaborate PGs of the E series, which appear to depress the immune system and to cause local bone changes with calcium secretion into the tumor area.

2367 ALTERED BILE ACID METABOLISM IN VEGETARIANS. (Eng.) Hepner, G. W. (Milton S. Hershey Medical Center, Pennsylvania State Univ., Hershey, Pa. 17033). *Am. J. Dig. Dis.* 20(10):935-940; 1975.

Bile acid kinetics were studied in ten healthy vegetarians and in ten matched controls. Each subject was given 20 μCi of [^{3}H]-labeled 2,4-cholic acid and 10 μCi of [^{14}C]-labeled 24-chenodeoxycholic acid dissolved in water. On the first, second, third and seventh days following administration, subjects were given 40 U cholecystokinin iv, and 2-4 ml concentrated bile was withdrawn from the duodenum. Samples were analyzed for pool sizes of cholic, chenodeoxycholic and deoxycholic acids and daily fractional turnover rates (DFTR) were calculated for cholic and chenodeoxycholic acids. The pool size of deoxycholic acid was significantly smaller in vegetarians (17.9 M/kg) than controls (28.9 M/kg). The DFTR of cholic acid was significantly less in vegetarians (21.5%) than in controls (33.0%). The decreased DFTR in vegetarians may be due to subtle differences in intestinal transit time or to differences in intestinal flora due to the dietary differences. This study demonstrates that meat may have an important effect on bile acid metabolism, and the author suggests that the differences in deoxycholic acid pools may be associated with the prevalence of bowel cancer in developed countries because of higher meat consumption.

2368 CELL SURFACE CHARGE AND REGULATION OF CELL DIVISION OF 3T3 CELLS AND TRANSFORMED DERIVATIVES. (Eng.) Adam, G. (Fachbereich Biologie, Universitat Konstanz, D-7750 Konstanz, West Germany); Adam, G. *Exp. Cell. Res.* 93(1):71-78; 1975.

2369 *IN VITRO* ANALYSIS OF THE CONTROL OF KERATINOCYTE PROLIFERATION IN HUMAN EPIDERMIS BY PHYSIOLOGIC AND PHARMACOLOGIC AGENTS. (Eng.) Flaxman, B. A. (Miriam Hosp., 164 Summit Ave., Providence, R.I. 02905); Harper, R. A. *J. Invest. Dermatol.* 65(1):52-59; 1975.

2370 MITOTIC AUTOREGULATION OF NORMAL AND ABNORMAL CELLS: ALTERNATIVE MECHANISMS FOR THE DERANGEMENT OF GROWTH CONTROL. (Eng.) Wheldon, T. E. (West of Scotland Health Boards, Dept. Clinical Physics and Bio-Engineering, 11 West Graham St., Glasgow G4 9LF, Scotland). *J. Theor. Biol.* 53(2):421-433; 1975.

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